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The Viscosity of Ideal Solutions

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The viscosity determination of artificially ideal solutions of glass spheres in a suspending medium with density equal to that of the solids in order to preclude sedimentation, done by Vand[1] and by his followers, Sweeny and Geckler[2], gave a decisive confirmation of Einstein's viscosity equation for infinite dilution

$$\eta_r = 1 + k\phi \text{ with } k = 2.5$$

where η_r is the relative viscosity and ϕ is the volume fraction occupied by N spheres of radius a in a volume V, $\phi = \frac{4}{3}\pi a^3 N/V$

The extention of Einstein's equation to a suspension of finite concentration proposed by Mooney[3],

$$\ln \eta_r = 2.303 \log \eta_r = 2.5\, z_v/(1-\lambda z_v)$$

where λ is a constant, the self-crowding factor, predicted only approximately by Mooney's theory, and z_v is the volume fraction of spheres, fits Vand's data as well as Sweeny and Geckler's data. It is noted here that since $z_v = \phi/(1+\phi)$, z_v becomes ϕ at infinite dilution where Mooney's equation tends to Einstein's equation. For actually ideal solutions, however, Einstein's equation fails and Arrhenius' equation[4] is frequently used:

$$\ln \eta_r = 2.303 \log \eta_r = Az_v$$

where A is a constant determined by the extrapolation of $\ln \eta_r/z_v$ at $z_v = 0$. Its determination, however, is difficult because the limit of precision of viscosity determination is reached. A general viscosity equation for infinite dilution derived later in this paper is

$$\eta_r = 1 + K_v(\eta_2/\eta_1)z_v$$

where η_2/η_1 is the viscosity ratio of the solute 2 to the solvent 1, and K_v is a constant throughout the concentration, determined easily as an arithmetic mean of the values $(1-z_v)(\eta-\eta_1)/z_v(\eta_2-\eta)$ at several finite concentrations, η being the viscosity of the solution at concentration z_v, so that K_v is accurate and trustworthy. The constant $K_v(\eta_2/\eta_1)$ has been found always to have a smaller value than 2.5 for the solute of low viscosity ratio, but its value comes near to 2 or more as the viscosity ratio becomes high.

In a case where the viscosity ratio is high and K_v keep constancy no longer against concentration, Mooney's equation having $(K_v)_0(\eta_2/\eta_1)$ in place of 2.5, namely:

$$\ln \eta_r = 2.303 \log \eta_r = (K_v)_0(\eta_2/\eta_1)z_v/(1-\lambda z_v)$$

where $(K_v)_0$ is the limiting value of K_v at $z_v = 0$, has been proved to be valid for the whole range of concentration.

Derivation of a New Equation at Infinite Dilution.—The hyperbolic form of the present author's fundamental formula for binary mixtures[5] is

$$\frac{(1-z_m)(\eta-\eta_1)}{z_m(\eta_2-\eta)} = \frac{k_2 a_2}{k_1 a_1} = K$$

where z_m is the molar fraction of component 2, a_1 and a_2 are the association degrees of components 1 and 2 respectively, k_1 and k_2 are the characteristic constants of components 1 and 2 respectively and K keeps constancy so long as no change occurs in the molecular association of either or both of components.

Now, between the molar fraction z_m and volume fraction z_v, there is the following relationship:

$$\frac{(1-z_m)M_1}{z_m M_2} = \frac{(1-z_v)d_1}{z_v d_2}$$

in which M_1 and M_2 are the molecular weights of components 1 and 2 respectively, and d_1 and d_2 are the densities of components 1 and 2 respectively, and therefore the similar hyperbolic form with respect to z_v:

$$\frac{(1-z_v)(\eta-\eta_1)}{z_v(\eta_2-\eta)} = K\frac{M_1 d_2}{M_2 d_1} = \text{a constant, say } K_v$$

is valid as well.

At very low concentration where z_v is small enough to be neglected as compared

1) V. Vand, *J. Phys.* and *Colloid Chem.*, **52**, 300 (1948).
2) K. H. Sweeny and R. D. Geckler, *J. Appl. Phys.*, **25**, 1135 (1954).
3) M. Mooney, *J. Colloid Sci.*, **6**, 162 (1951).
4) S. Arrhenius, *Z. Phys. Chem.* **1**, 285 (1887).
5) T. Ishikawa, This Bulletin, **4**, 5 (1929).

to 1, the above formula can be reduced to

$$\frac{\eta-\eta_1}{z_v(\eta_2-\eta)}=K_v$$

from which it follows:

$$\frac{\eta}{\eta_1}=\frac{1+K_v(\eta_2/\eta_1)z_v}{1+K_v z_v}$$

Since K_v has been found to be always less than or very rarely equal to unity in cases where the component having higher viscosity between the two is taken as component 2 (see Table I), the second term of the denominator can be neglected as compared to 1, hence the following general equation results:

$$\frac{\eta}{\eta_1}=\eta_r=1+K_v(\eta_2/\eta_1)z_v$$

In Table I are shown K_v, (η_2/η_1) and $K_v(\eta_2/\eta_1)$ of 18 kinds of ideal mixture quoted from as many viscosity data with respect to z_v as are found in the literature. Each K_v is an arithmetic mean of these at all the concentrations determined. Out of these pairs three cases, the one having the lowest viscosity ratio, benzene-carbon tetrachloride, and the other two having the highest viscosity ratios, toluene-ethyl benzoate and toluene-diphenyl, are exemplified in Tables II, III and IV.

The solutions of naphthalene and of diphenyl in benzene and in toluene have often been used as typical ideal solutions since Kendall and Monroe[6] made the viscosity measurements on these solutions in order to test their own cube-root formula[7], $\eta^{1/3}=(1-z_m)\eta_1^{1/3}+z_m\eta_2^{1/3}$ but they obtained different values 0.0225 and 0.0183 for the solutional viscosity of naphthalene in benzene and in toluene respectively, and 0.0344 and 0.0282 for that of diphenyl in these solvents respectively. The present author[8], by using his own formula described above, obtained concordant values in both solvents, 0.02653 for naphthalene and 0.05413 (revised value 0.05509) for diphenyl. Van der Wyk[9] reported that he had obtained concordant values in both

TABLE I

K_v, (η_2/η_1) AND $K_v(\eta_2/\eta_1)$ VALUES OF IDEAL SOLUTIONS

Component 1	Component 2	t°C	η_1	η_2	η_2/η_1	K_v	$K_v\times(\eta_2/\eta_1)$	Observer
Benzene	Carbon tetrachloride	60	0.00391	0.00582	1.488	0.737	1.097	Thorpe and Rodger[10]
Benzene	Bromobenzene	18.2	0.00654	0.01129	1.726	0.926	1.598	Yajnik et al[11]
Benzene	Ethyl benzoate	25	0.006051	0.01991	3.290	0.430	1.415	Kendall and Monroe[7]
Benzene	Naphthalene	25	0.006048	(0.02653)	4.387	0.347	1.522	Kendall and Monroe[6]
Benzene	Diphenyl	25	0.006051	(0.05509)	9.104	0.172	1.566	Kendall and Monroe[6]
Toluene	Chlorobenzene	20	0.00447	0.00709	1.586	1.063	1.681	Yajnik et al[11]
Toluene	Bromobenzene	25	0.00447	0.00880	1.969	0.877	1.725	Yajnik et al[11]
Toluene	Ethyl benzoate	25	0.005520	0.01990	3.605	0.446	1.609	Kendall and Monroe[7]
Toluene	Naphthalene	25	0.005526	(0.02653)	4.716	0.322	1.519	Kendall and Monroe[6]
Toluene	Diphenyl	25	0.005520	(0.05509)	9.980	0.168	1.677	Kendall and Monroe[6]
Ethyl ether	Benzene	25	0.00230	0.00599	2.604	0.494	1.287	Linebarger[12]
Acetone	Benzene	25	0.00315	0.005758	1.823	0.486	0.886	Fischler[13]
Ethyl alcohol	*iso*-propyl alcohol	25	0.01080	0.02048	1.896	0.595	1.128	Parks and Kelley[14]
Methyl alcohol	Nitrobenzene	25	0.005608	0.018126	3.232	0.303	0.979	Fischler[13]
Cyclohexane	Decaline	25	0.009287	0.02171	2.338	0.539	1.259	MacFarlane and Wright[15]
Ethylene chloride	Ethylene bromide	25	0.007812	0.01613	2.065	0.646	1.334	MacFarlane and Wright[15]
Benzene	Benzyl benzoate	25	0.006044	0.08454	13.99	0.132 ~0.225	1.847	Kendall and Monroe[7]
Toluene	Benzyl benzoate	25	0.005520	0.08450	15.31	0.124 ~0.215	1.898	Kendall and Monroe[7]

6) J. Kendall and K. P. Monroe, *J. Am. Chem. Soc.*, **43**, 115 (1921).
7) J. Kendall and K. P. Monroe, ibid., **39**, 1787 (1917).
8) T. Ishikawa, This Bulletin, **5**, 47 (1930).
9) A. J. A. van der Wyk, *Nature*, **138**, 845 (1936).
10) T. E. Thorpe and M. D. Rodger, *J. Chem. Soc.*, **71**, 360 (1897).
11) N. A. Yajnik, M. D. Bhalla, R. C. Talwar and M. A. Soofi, *Z. phys. Chem.*, **118**, 305 (1925).
12) C. E. Linebarger, *Am. J. Sci.*, [4] **2**, 331 (1896).
13) J. Fischler. *Z. Elektrochem.*, **19**, 126 (1913).
14) G. S. Parks and K. K. Kelley, *J. Phys. Chem.*, **29**, 727 (1925).
15) W. MacFarlane and R. Wright, *J. Chem. Soc.*, **1933**, 114.

TABLE II

BENZENE(1)-CARBON TETRACHLORIDE(2), 60°C

z_v	z_m	η_{obs}	K_v	η_{calc}	S_v	λ
0.000	0.0000	0.003905				
0.208	0.1949	0.00422	0.738	0.00422	1.11	−9.33
0.414	0.3944	0.00456	0.730	0.00456	1.12	−4.66
0.656	0.6377	0.00503	0.743	0.00503	1.10	−2.81
1.000	1.0000	0.005835	0.737		1.11	

N. B. $K_v(\eta_2/\eta_1)=1.097$, $2.303\log(\eta_2/\eta_1)/S_v=0.358$

TABLE III

TOLUENE(1)-ETHYL BENZOATE(2), 25°C

z_v	z_m	η_{obs}	K_v	η_{calc}	S_v	λ
0.0000	0.0000	0.005520				
0.1951	0.1524	0.006845	(0.4187)	0.006924	1.202	−2.35
0.4256	0.3546	0.009076	0.4434	0.009094	1.170	−0.89
0.6964	0.6299	0.01279	0.4448	0.01279	1.207	−0.48
0.7890	0.7349	0.01452	0.4474	0.01451	1.219	−0.40
0.8798	0.8442	0.01655	0.4498	0.01653	1.229	−0.33
1.0000	1.0000	0.01990	0.4464		1.205	

N. B. $K_v(\eta_2/\eta_1)=1.609$, $2.303\log(\eta_2/\eta_1)/S_v=1.064$

TABLE IV

TOLUENE(1)-DIPHENYL(2), 25°C

z_v	z_m	η_{obs}	K_v	η_{calc} Ishikawa $\eta_2=0.05509$	Kendall $\eta_2=0.0282$	van der Wyk $\eta_2=0.0374$ $\eta_{12}=0.0154$	S_v	λ
0.000	0.0000	0.005520						
0.186	0.1398	0.007335	0.166	0.007353	0.007369	0.007334	1.62	−0.52
0.282	0.2197	0.008587	0.168	0.008588	0.008584	0.008547	1.65	−0.25
0.348	0.2761	0.009627	0.169	0.009599	0.009528	0.009552	1.67	−0.14
			0.168				1.65	

N. B. $K_v(\eta_2/\eta_1)=1.677$, $2.303\log(\eta_2/\eta_1)/S_v=1.395$

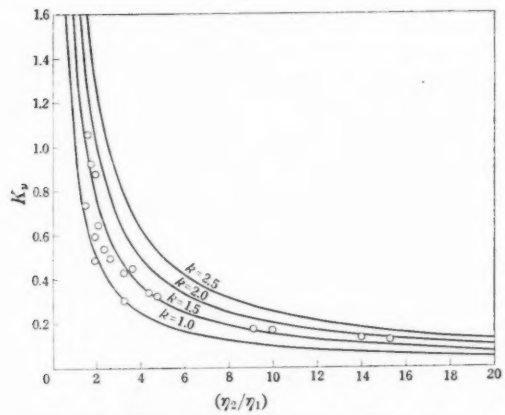

Fig. 1. The relation between K_v and (η_2/η_1) of ideal solutions.

solvents, 0.0219~0.0224 for naphthalene and 0.03740 for diphenyl by the use of his formula

$$\log\eta=(1-z_m)^2\log\eta_1+2z_m(1-z_m)\log\eta_{12}+z_m^2\log\eta_2$$

In Table IV are also shown for reference the calculated viscosity values due to Kendall and Monroe's formula and van der Wyk's formula.

Fig. 1 shows a plot of K_v against (η_2/η_1) for the results given in Table I. Let the product $K_v(\eta_2/\eta_1)$ be denoted by k as the same signature as the above, mixtures or solutions having the same values as k will take positions on a hyperbolic curve $K_v(\eta_2/\eta_1)=k$. In Fig. 1 solid lines represent $k=1.0$, 1.5, 2.0 and 2.5. As seen from the positions of the points, it appears that the higher (η_2/η_1) becomes, the less K_v decreases, and that this tendency undoubtedly signifies the approach of k to 2 or more, yet its limit can not be determined by this method of deduction.

Cases where K_v varies with Concentration. — Kendall and Monroe[7] took the

following four systems: benzene-ethyl benzoate, toluene-ethyl benzoate, benzene-benzyl benzoate, and toluene-benzyl benzoate, as chemically indifferent mixtures having great differences in viscosities and molecular weights of both components, and ascertained the validity of their cube-root formula with good result for the first two mixtures, but with bad result for toluene-benzyl benzoate contrary to their presupposition that this system was regarded to be a normal mixture as judged from the fact of there being no appreciable thermal effect and volume change on mixing.

Since then the data of this system have been used for the verification of ideal viscosity formulas. The present author[16] considered, however, that if component 2 suffers molecular dissociation in solution, K of his formula keeps no more constancy, and that if observed K values lie on a straight line, the extrapolation values at $z_m=0$, and $z_m=1$ can be obtained with ease and accuracy, and he obtained for this mixture 0.250 at $z_m=0$, and 0.390 at $z_m=1$. Lederer[17] proposed an ideal viscosity formula in the form

$$\log \eta = \frac{(1-z_m)\log \eta_1}{1-z_m+z_m/S} + \frac{z_m \log \eta_2/S}{1-z_m+z_m/S}$$

where S is the ratio of association degrees of component 2 to that of component 1. His idea of an association molecule of liquids is a molecule similar to a chemically fast-combined molecule. This is quite different from the present author's idea expressing an associated molecule as a group of single molecules interchangable with singly existing molecules, which is in accord with an idea of a cybotactic group proposed a short time later by Stewart[18]. The value of S obtained by Lederer for this mixture is 1.197[19] which is an arithmetic mean of 1.250, 1.217, 1.190, 1.166 and 1.160 at respective concentrations in their order in Table V. Van der Wyk[9] also treated this mixture with his formula, and obtained, by putting $\eta_{12}=0.0275$, fairly concordant values with experimental data (see columns 9 and 10 in Table V).

As stated above, K for this mixture varies linearly with z_m, so K_v varies with z_v and is found to be expressible by a linear equation with $(K_v)_0=0.124$ and $(K_v)_1=0.215$. Similarly for benzene-benzyl benzoate mixture we get $(K_v)_0=0.132$ and $(K_v)_1=0.225$. These values are tabulated in Table I, and the accuracy of these values can be acknowledged from η calculated from thus obtained K_v in column 6 in Tables V and VI.

If there be among the dissociated molecules the crowding effect postulated by Mooney's theory, the following modified form of his equation having $(K_v)_0(\eta_2/\eta_1)$ in place of 2.5 would be valid for these cases:

$$\ln \eta_T = 2.303 \log \eta_T = (K_v)_0(\eta_2/\eta_1) z_v/(1-\lambda z_v)$$

A few trials have given satisfactory results to this presumption: $(K_v)_0(\eta_2/\eta_1)=1.898$ and $\lambda=0.300$ for toluene-benzyl benzoate, and $(K_v)_0(\eta_2/\eta_1)=1.847$ and $\lambda=0.296$ for benzene-benzyl benzoate, and the calculated viscosity values by making use of these equations are tabulated in column 7 in Tables V and VI.

A severe test which must be made here is the viscosity data of the system, petroleum-non-plastic asphalt ($\eta_2/\eta_1=10^6$ at 15°C), observed by Saal and Koens and studied by Cragoe[20] for the confirmation of the latter's formula for ideal mixtures:

$$L_m=(1-z)L_1+zL_2$$

where z is the weight fraction of component 2, L with suffixes 1, 2 and m (the mixture) are defined by $\eta=Ae^{\frac{B}{L}}$, and A and B are constants, $A=5\times10^4$ and $B=1000 \ln 20$. Cragoe applied his formula with success not only to mixtures of viscosity ratios less than 100, but also to this system, for which other formulas such as those of Arrhenius[4], Bingham ($1/\eta=(1-z_v)/\eta_1+z_v/\eta_2$)[21], and Kendall and Monroe[7] failed.

For this mixture the variation of K_v values displays a strongly sagged curve not to be satisfactorily expressed by any simple functional form, so we obtain at first 5.45 as the extrapolation value of the usual form η_{sp} (specific viscosity)$=kz_v$, by assuming a straight line to pass the values at two low concentrations, $z_v=0.224$ and 0.464, and then put this or a near value in the modified Mooney's equation to find λ to have a constant against z_v. By a few trials the following equation has been found to fit the observed values:

$$\ln \eta_T = 2.303 \log \eta_T = 5.40 z_v/(1-0.608 z_v)$$

16) T. Ishikawa, This Bulletin, **4**, 288 (1929).
17) E. L. Lederer, *Proc. World Petroleum Congress, London*, **2**, F. 526 (1933).
18) G. W. Stewart, *Rev. Mod. Phys.*, **2**, 116 (1930); *Koll. Z.*, **67**, 130 (1934).
19) E. L. Lederer, *Nature*, **139**, 27 (1937).
20) C. S. Cragoe, *Proc. World Petroleum Congress, London*, **2**, F. 529 (1933).
21) E. C. Bingham, *Am. Chem. J.*, **35**, 195 (1906).

TABLE V

TOLUENE(1)-BENZYL BENZOATE(2), 25°C

z_v	z_m	η_{obs}	K_v	$(K_v)_{calc}$	Ishikawa eq. with $(K_v)_{calc}$	Modified Mooney eq. $\lambda=0.300$	Lederer eq. $S=1.197$	Van der Wyk eq. $\eta_{12}=0.0275$	S_v
0.0000	0.0000	0.005520		(0.124)					
0.3558	0.2367	0.01183	0.157	0.156	0.01179	0.01175	0.01154	0.0115	1.686
0.5693	0.4261	0.02015	0.172	0.176	0.02043	0.02031	0.01993	0.0201	1.463
0.7682	0.6502	0.03614	0.191	0.194	0.03642	0.03670	0.03624	0.0364	1.498
0.8694	0.7890	0.05080	0.202	0.203	0.05070	0.05143	0.05104	0.0515	1.526
0.9416	0.9002	0.06660	0.212	0.210	0.06650	0.06660	0.06707	0.0667	1.541
1.0000	1.0000	0.08450		(0.215)					1.507

N. B. $(K_v)_0(\eta_2/\eta_1)=1.898$, $2.303\log(\eta_2/\eta_1)/S_v=1.811$

TABLE VI

BENZENE(1)-BENZYL BENZOATE(2), 25°C

z_v	z_m	η_{obs}	K_v	$(K_v)_{calc}$	Ishikawa eq. with $(K_v)_{calc}$	Modified Mooney eq. $\lambda=0.296$	S_v
0.0000	0.0000	0.006044		(0.132)			
0.3308	0.1886	0.01196	0.165	0.163	0.01190	0.01190	1.416
0.5991	0.4124	0.02301	0.185	0.188	0.02326	0.02319	1.455
0.7484	0.5832	0.03584	0.205	0.202	0.03550	0.03567	1.434
0.8844	0.7827	0.05478	0.214	0.214	0.05477	0.05523	1.506
0.9478	0.8952	0.06883	0.220	0.220	0.06885	0.06884	1.535
1.0000	1.0000	0.08454		(0.225)			1.467

N. B. $(K_v)_0(\eta_2/\eta_1)=1.847$, $2.303\log(\eta_2/\eta_1)/S_v=1.755$

TABLE VII

PETROLEUM(1)-NON-PLASTIC ASPHALT(2), 15°C

z	z_v	η_{obs}	$(\eta_r)_{obs}$	K_v	$(\eta_r)_{calc}$ Modified Mooney eq. $\lambda=0.608$	$(\eta_r)_{calc}$ Lederer eq. $S_v=2.52$	$(\eta_r)_{calc}$ Cragoe eq.	S_v
0.00	0.000	1.31	1.000					
0.25	0.224	5.30	4.046	1.097×10^{-5}	4.055	4.122	4.450	2.56
0.50	0.464	39.6	30.23	5.564×10^{-5}	32.77	33.94	39.54	2.63
0.75	0.724	1850	1412	56.01×10^{-5}	1078	1133	1328	2.36
1.00	1.000	1.26×10^{6}	9.618×10^{5}		9.582×10^{5}	9.670×10^{5}		2.52

N. B. $(K_v)_0(\eta_2/\eta_1)=5.40$, $2.303\log(\eta_2/\eta_1)/S_v=5.47$

the calculated values being tabulated in column 6 in Table VII with reference to Cragoe's results. $(K_v)_0=0.561\times10^{-5}$ obtained by putting $(K_v)_0(\eta_2/\eta_1)=5.40$ may be an acceptable value as supposed from a plot of K_v against concentration. Furthermore, $\lambda=0.608$ is quite coincident with the hydrodynamic interaction constant 0.609 derived by Vand[22].

It is noted that another method of finding the shape factor k and the crowding factor λ is the proposition recommended by Maron et al.[23] that a graph $z_v/\log\eta_r$ *vs* z_v should be linear.

Applicability of Modified Mooney's Equation.—Since Lederer's formula is analogous in form to the author's formula, it can be transformed into the following hyperbolic form with respect to z_v:

$$\frac{(1-z_v)(\log\eta-\log\eta_1)}{z_v(\log\eta_2-\log\eta)} = \text{a constant, say } 1/S_v$$

or

$$\log\eta_r=\frac{\{\log(\eta_2/\eta_1)/S_v\}z_v}{1-(1-1/S_v)z_v}$$

and therefore

$$\ln\eta_r=2.303\log\eta_r=\frac{2.303\{\log(\eta_2/\eta_1)/S_v\}z_v}{1-(1-1/S_v)z_v}$$

This transformed formula, when it is applied to the above taken petroleum-non-plastic asphalt system with constant

22) V. Vand, *J. Phys.* and *Colloid Chem.*, **52**, 277 (1948).
23) S. H. Maron, B. P. Madow and I. M. Krieger, *J. Colloid Sci.*, **6**, 584 (1951).

$S_\nu=2.52$, is found to be available with accuracy equal to the modified Mooney's equation (see column 7 in Table VII), so that these two equations appear to be identical. If the identity is generally true, the numerical values of the corresponding factors $2.303\log(\eta_2/\eta_1)/S_\nu$ and $(K_\nu)_0(\eta_2/\eta_1)$ should coincide with each other, and to such solutions only the applicability of the modified Mooney's equation should be restricted, because its application to solutions of low viscosity ratios (K_ν=constant) gives λ negative inconstant values as seen from λ values in Tables II, III and IV.

Examinations of all the data taken in this paper lead us to the conclusions (1) that for solutions of low viscosity ratios (K_ν=constant) e.g., benzene-carbon tetrachloride, toluene-ethyl benzoate, and toluene-diphenyl (see N. B. in Tables II, III and IV), $2.303\log(\eta_2/\eta_1)/S_\nu$ is always less than $(K_\nu)_0(\eta_2/\eta_1)$, (2) that for solutions of appreciably high viscosity ratios in which slight molecular dissociation occurs e.g., benzene-benzyl benzoate and toluene-benzyl benzoate (see N. B. in Tables V and VI), the constancy of S_ν is not favorable and, if we take the mean of such S_ν values, $2.303\log(\eta_2/\eta_1)/S_\nu$ becomes slightly less than $(K_\nu)_0(\eta_2/\eta_1)$ to make the validity of Ledere's formula inferior to the modified Mooney's equation, and (3) that for solutions of extemely high viscosity ratios where the molecular dissociation follows and consequently geometric crowding action and hydrodynamic interaction occur among the dissociated molecules, e. g., petroleum-non-plastic asphalt (see N. B. in Table VII), these two factors coincide with each other and $(1-1/S_\nu)$ will tend to λ in consequence.

From what has been discussed above a modified form of Mooney's equation with $(K_\nu)_0(\eta_2/\eta_1)$ in place of 2.5:

$$\ln\eta_T=2.303\ln\eta_T=(K_\nu)_0(\eta_2/\eta_1)z_\nu/(1-\lambda z_\nu)$$

may be regarded as a viscosity formula for ideal solutions of high viscosity ratios.

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The Two Stable Isomers of Chlorocyclohexane

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Twenty years ago Canals et al.[1] studied the Raman spectra of chlorocyclohexane and found the two bands which may be assigned to the C-Cl stretching vibration. As it is now well established that the structure of cyclohexane ring is the chair form, it can be easily understood that the two bands are due to the two isomers which may be represented by the usual symbols e and a. Hassell and Viervoll[2] concluded from their electron diffraction data that the molecules of chlorocyclohexane are almost all of the e-form in the vapor state. Larnaudie[3], however, pointed out the coexistence of the e- and the a-form in the vapor as well as in the liquid state, since the two characteristic bands for the C-Cl stretching vibration were observed in both states in his infrared investigations. It seems likely to be interesting to determine the form of the more stable isomer and the energy difference between these two isomers. For these purposes we carried out the following researches.

Experimental

Preparation and Purification of Chlorocyclohexane. — According to Markownikoff[4] cyclohexanol was heated at 100°C in a sealed glass tube with concentrated hydrochloric acid solution for five hours. The oily substance which had separated in the tube was distilled with

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1) E. Canals, M. Mousseron, R. Granger and J. Gastaud, *Bull. soc. chim. France*, **4**, 2048 (1937).

2) O. Hassel and H. Viervoll, *Tids. Kjemi, Bergvesen Met.*, **5**, 35 (1943).

3) M. Larnaudie, *Compt. rend.*, **235**, 154 (1952); *J. phys. radium*, **15**, 650 (1954).

4) W. Markownikoff, *Ann.*, **302**, 19 (1898).

TABLE I

VIBRATIONAL SPECTRA OF CHLOROCYCLOHEXANE

Raman*		Infrared**		Configuration
liquid (cm^{-1})	solid (cm^{-1})	liquid (cm^{-1})	solid (cm^{-1})	
200 (1)				
259 (1)				
339 (9)	340 (3)			e
437 (3)	435 (1)	435.2(w)		e
		472.4(w)		
509 (3)	508 (0)	512 (m)	510.5(s)	e
560 (6)		559 (m)		a
			628 (vw)	e
686 (4)		684.5(s)		a
			711.7(w)	e
734 (8)	726 (4)	731 (vvs)	728 (vvs)	e
		789 (vw)	790 (w)	e
808 (5)		807 (m)		a
822 (5)	821 (2)	817 (vs)	817 (vs)	e
			~845 (w)sh	e
852 (5)	851 (2)	852 (m)	850.5(m)	e
		858 (m)		a
		863 (m)		a
		~868 (m)		a
890 (2)		889 (s)	888.5(vs)	e
		920 (w)	920.5(w)	e
		972 (vw)	972 (vw)	e
992 (6)	993 (3)	993 (vs)	994 (s)	e
		1014 (m)		a
1027 (6)	1027 (2)	1029 (w)	1025 (w), 1031 (w)	e
1051 (2)	1053 (1)	1051 (vw)	1050 (w)	e
		1070 (vw)	1068 (vw), 1075 (vw)	e
		1089 (vw)	1088 (w)	e
		1098 (w)		a
		1132 (w)	1134 (m)	e
		1145 (w)		a
		1184 (w)	1186 (w)	e
1215 (2)	1216 (0)	1215 (s)	1216 (s)	e
1263 (6)	1261 (1)	1258 (m), 1267 (s)	1256 (s), 1267 (m)	e, e
1297 (0)		1301 (vw)	1300 (w)	e
			1323 (m)	e
1337 (2)		1340 (m)	1332 (m)	e
			1339 (m)	e
1447 (7)	1443 (2)	1448 (vs)	1446 (s)	
2865 (10b)		2617 (vw)		
2904 (9b)		2675 (w)	2670 (s)	
2951 (10b)	2945 (2b)	2860 (vs)	2860 (s)	
		2930 (vs)	2930 (vs)	

* For the Raman spectra the numbers shown in the brackets are the visually estimated relative intensities.

** The infrared spectra were not investigated below 430 cm^{-1} in the liquid and below 500 cm^{-1} in the solid. The letters shown in the brackets mean the relative intensities. v=very, s=strong, m=medium and w=weak. sh=shoulder.

TABLE II

INFRARED SPECTRA OF THE VAPOR OF CHLOROCYCLOHEXANE

wave number	configuration	wave number	configuration	wave number	configuration
~502		864 (m)	a	1160 (vw)	
510.0(m)	e				
516		872 (m)	a	1180 (vw)	
~566 (m,b)	a	887		1183 (vw)	
		893 (s)	e		
682.6				1214	
690.2(m)	a	992		1220 (m)	e
697.0		997 (s)	e	1225	
		1001			
734.4				1265 (s)	
740.3(vs)	e	1017 (m)	a	1269 (s)	
745.8				1273 (s)	
		1024 (m)		1280 (s)	
~796 (vw)	e			1352 (m)	
		1079 (w)		1459 (vs)	
814.5					
820 (s)	e	1094		2176 (vw)	
826		1103 (w)	a	2510 (vw)	
		1111		2680 (w)	
854 (m)	e			2890 (vs)	
		1129		2950 (vs)	
		1140 (w)	e		

steam, washed with water, and dried over calcium chloride. The product was further purified by fractional distillation. (b.p. 142.0~142.3°C).

Vibrational Spectra.—The Raman spectra of this compound were observed for the liquid and the solid state with the same spectrograph as in our earlier works[5]. The infrared spectra were obtained for the vapor (ca. 120°C), liquid, and solid states, by use of a Perkin-Elmer Spectrometer Model 112 equipped with sodium chloride and potassium bromide prisms. The wave number readings for both spectra are shown in Tables I and II.

The Raman spectra of the liquid obtained by Kohlrausch et al.[6] agree with our data, except the doublets at 808 and 822 cm^{-1} and some additional weak bands. The data obtained by Canals et al.[1] are incomplete and disagree with our results at several points.

Measurements of the Integrated Intensities in the Vapor State.—The integrated intensities of the two bands at 689 cm^{-1} and 741 cm^{-1} were obtained with a potassium bromide prism. The slit width used was 0.280 mm and the spectral slit widths were 3.3cm^{-1} at 700cm^{-1} and 4.0cm^{-1} at 750cm^{-1}. The gas absorption cell was made of a glass cylinder with two potassium bromide windows and was heated electrically by a nichrome wire. In order to avoid the condensation of the vapor on the windows short glass cylinders heated electrically were attached in front of the windows. The path length of the cell was 6 cm.

Measurements were made at the two temperatures 117°C and 200°C*. Atmospheric carbon dioxide was removed from the optical path as far as possible. Such a high temperature, at which the measurements were made, causes emission bands of the compound with noticeable intensities. The radiation intensity I_b of the high temperature cell and the intensity I_d of the emission band of the sample at frequency ν were measured by placing a non-reflecting shutter made of a piece of glass and a sheet of black paper behind the cell. For the evaluation of the absorbance $\log_{10} I_0/I$, and I_0 and I should be

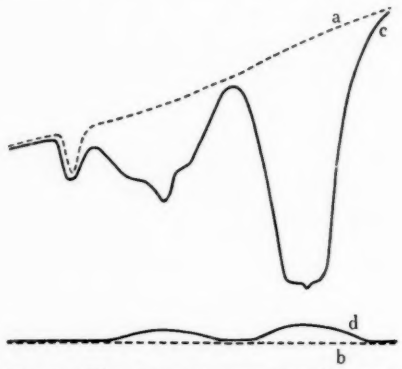

Fig. 1. The absorption curves a, b, c and d are used for obtaining I_a, I_b, I_c, and I_d, respectively.

5) a) K. Kozima and T. Yoshino, *J. Am. Chem. Soc.*, **75**, 166 (1953); b) K. Kozima, K. Sakashita and S. Maeda, ibid., **76**, 1965 (1954).

6) K. W. F. Kohlrausch and W. Stockmair, *Z. physik. Chem.*, **B31**, 382 (1936).

* Above 250°C the shapes of the bands change gradually and new bands appear.

TABLE III
RATIO OF INTEGRATED INTENSITIES (VAPOR)

	No. of Experiments	$\bar{\nu}_{max}$	Ratio of integrated intensities obtained by weighing
$t=117°C$	1	689.5 cm^{-1} 741.5	0.347
	2	690.0 741.2	0.347
	3	689.7 741.3	0.349
	Mean	689.7 741.3	0.348
$t=200°C$	1	689.0 740.6	0.376
	2	689.5 740.0	0.380
	3	689.3 739.7	0.371
	Mean	689.3 740.1	0.376

taken as

$$I_0=I_a-I_b,\ I=I_c-I_d$$

where I_0 and I have their usual meanings, and I_a and I_c are the monochromatic intensities of radiation after passage through the vacant cell and the cell containing the sample at a high temperature, respectively. The absorption curves obtained are shown in Fig. 1.

The area-intensities were obtained by the graphical integration of the absorbances over the frequency interval from 641 cm^{-1} to 790 cm^{-1}, which sufficiently covers the range of about four times the half intensity width for each band. The integration outside this interval may be neglected without noticeable influences on the evaluation of the relative intensities, because the absorption on the wings of the band decreases rather rapidly; that is to say, for the band near 689 cm^{-1} its absorbance at 641 cm^{-1} is smaller than 1/20 of that at the peak and for the other band near 741 cm^{-1} the decrement of that at 790 cm^{-1} is more serious. The absorption curve near 710 cm^{-1}, which consists of the two bands overlapping each other at their wings, was divided into the original bands, so that the bands have the most reasonable shapes. The band near 741 cm^{-1} overlaps slightly with that near 820 cm^{-1}, which includes a very weak band near 796 cm^{-1}. This overlapping was also treated by the same method as described above. The correction for finite slit widths may be neglected, because of the broadness of the bands. The results obtained are shown in Table III.

Measurements of the Integrated Intensities in the Solution.—The integrated intensities of the two bands near 685 cm^{-1} and 732 cm^{-1} were obtained at 25°C in the solution, carbon disulfide and methyl acetate being used as solvents. The prism and the slit width were the same as those employed in the above intensity measurement. The thickness of the cell used was 0.2 mm. There were no remarkable differences between the ratios of the integrated intensities obtained by the graphical integration and those obtained by Ramsay's method[7] of direct integration corrected for finite slit widths. The results are listed in Table IV.

Discussion

As shown in Table I the vibrational spectra in the solid state differ clearly from those in the liquid state; viz., some of the relatively strong bands observed in the liquid spectra disappear in the solid spectra.

For the C-Cl stretching vibration two characteristic bands were observed at 684.5 and 731 cm^{-1} in the liquid state, while in the solid state the former disappeared and the latter persisted. Therefore, it is concluded that the dynamic equilibrium of the two isomers, which exists in the liquid state, breaks down in the course of the crystalization of the compound and only one isomer persists in the solid state. On the other hand the infrared spectra in the vapor state, shown in Table II, correspond well with those in the liquid state.

7) D. A. Ramsay, *J. Am. Chem. Soc.*, **74**, 72 (1952).

TABLE IV
THE RATIOS OF INTEGRATED INTENSITIES (SOLUTION)

temperature: 25°C
solvent: carbon disulfide

No. of experiments	mol./l.	$\bar{\nu}$ cm^{-1}	$\log_e(T_0/T)$	$\Delta\bar{\nu}^0_{1/2}$ cm^{-1}	A	A_a/A_e	A'_a/A'_e (graphical)
1	0.36	685.8 733.2	0.504 1.52	6.5 7.2	4.8_5 $17._1$	0.28	0.34
2	0.32	686.0 733.5	0.445 1.39	7.0 7.4	4.6_4 $16._1$	0.29	0.33
	mean	685.9 733.4				0.29	0.34
solvent:	methyl acetate						
1	0.55	684.8 731.8	0.532 1.94	8.1 8.6	6.5_1 $26._4$	0.25	0.26
2	0.40	684.6 731.5	0.445 1.64	8.0 8.4	5.3_8 $21._7$	0.25	0.25
	mean	684.7 731.7				0.25	0.26

The cell thickness for both solvents is 1.96×10^{-2} cm. The slit widths are 3.1 cm^{-1} at 686 cm^{-1}, 3.8 cm^{-1} at 733.5 cm^{-1} and 3.7 cm^{-1} at 731.5 cm^{-1}. A is the true integrated absorption intensity given by

$$A = K\log_e(T_0/T)_{\nu(\max)}\cdot\Delta\bar{\nu}^0_{1/2}$$

where the letters have their usual meanings. A_a/A_e shown in the table denotes the intensity ratio thus obtained and A'_a/A'_e the same ratio obtained by graphical integration. As for the carbon disulfide solution, agreements of the results obtained by both methods are not quite satisfactory, but the difference does not seriously alter the value of ΔE. ΔE for this solvent given in Table V is the mean value for both methods.

Therefore, the two isomers also coexist in the vapor state as pointed out by Larnaudie[3].

As has been shown by Larnaudie, the two C-Cl bands observed in the vapor state can confidently be identified with the e- and the a-form by the consideration of their band envelopes, respectively, based on the results obtained by Badger and Zumwalt[8]. The band at 740.3 cm^{-1} is assigned to the e-form and that at 690.2 cm^{-1} to the a-form. Since the band at 740.3 cm^{-1} in the vapor state corresponds to that at 728 cm^{-1} in the solid state, the isomer which persists in the solid state is the e-form. Among the relatively strong bands which appeared in the liquid and the vapor spectrum, the bands which persist in the solid state are assigned to the e-form and the other bands to the a-form as shown in Tables I and II.

It is possible to determine the energy difference between the two isomers by the measurement of the ratio of the integrated intensities of the two bands exhibited by the a- and the e-form, respectively, by use of the following formula:

$$A_a/A_e = \alpha_a N_a/\alpha_e N_e = (\alpha_a/\alpha_e)(f_a/f_e) \times\exp.(-\Delta E/RT)$$

where $A=\int\log_e(I_0/I)d\nu$ is the integrated intensity, $\Delta E=E_a-E_e$ the energy difference, α the molecular absorption coefficient, N the number of molecules, f the partition function, and the suffixes a and e refer to the a- and the e-form, respectively. In order to eliminate the unknown quantity $(f_a/f_e)(\alpha_a/\alpha_e)$, the measurements must be made at two temperatures at least. The ratio of the partition functions f_a/f_e is taken to be constant, because of the narrow range of temperature. The band at 740 cm^{-1} of the e-form and that at 690 cm^{-1} of the a-form are adequate for the purpose.

The results obtained for the vapor state at the temperatures 117° and 200°C are shown in Table III, and the energy difference thus obtained is about 0.34 kcal./mole, the e-form being the more stable isomer.

The energy differences in the dilute solutions can be estimated from the ratios of the integrated intensities shown in Table IV, if the values of (α_a/α_e) and (f_a/f_e) in the solution are assumed to be the same as those in the vapor state. The energy differences obtained for the solutions of carbon disulfide and methyl acetate are shown in Table IV. In these

8) R. M. Badger and L. R. Zumwalt, *J. Chem. Phys.*, **6**, 711 (1938).

solutions the e-form is also the more stable isomer.

As has been reported in our previous article[5b], the two mutually convertible isomers of *trans*-1,2-dichlorocyclohexane represented by the usual symbols as (e,e) and (a,a) are in a dynamic equilibrium for the various states, except for the solid state. By the studies on the electric dipole moment of the compound the energy difference between the isomers has been determined. For the reason mentioned below the energy difference between the isomers for both compounds should be determined for the same state. Therefore, the difference between the energy of the (e,e)-form and that of the (a,a)-form, $E_{ee}-E_{aa}$, was determined for the vapor, carbon disulfide solution and methyl acetate solution, by use of the same method as that used for the monochloro derivative. The results are listed in Table V.

TABLE V
THE ENERGY DIFFERENCES BETWEEN TWO MUTUALLY CONVERTIBLE ISOMERS
(inverted isomers)

State	$\Delta E=E_a-E_e$ chloro-cyclohexane kcal./mole	$\Delta E=E_{ee}-E_{aa}$ *trans*-1,2-dichloro-cyclohexane kcal./mole
vapor	0.34	0.61
CS_2 soln.	0.33	0.17
CH_3COOCH_3 soln.	0.44	−0.43

The energy difference between the mutually convertible isomers in the vapor state may be due to the difference between the intramolecular potential energies which are taken to be the sums of the potential energies of various pairs of non-bonded atoms.

Based upon this point of view, the following relation is obtained:

$$E_{ee}-E_{aa}+2(E_a-E_e)=E_g-E_t$$

where $E_{ee}-E_{aa}$ and E_a-E_e have the meanings mentioned above and E_g-E_t is the energy difference between the *gauche* and the *trans* form of 1,2-dichloroethane. The derivation of this formula will be given in an Appendix.

With this formula and the values for the vapor state listed in Table V, we find that $E_g-E_t=1.29$ kcal./mole. The value is in good agreement with that directly determined for the vapor state of 1,2-dichloroethane[9]. By use of the values for the solutions shown in the table, we find that $E_g-E_t=0.83$ kcal./mole for the dilute carbon disulfide solution and $E_g-E_t=0.45$ kcal./mole for the dilute methyl acetate solution.

These values, compared with the experimental data[10], seem to be reasonable, when taking into account the assumption made for the measurement in the solution. The reasons why the formula is available for the solution may be attributed to the facts that the variations of $E_{ee}-E_{aa}$ and E_g-E_t with the solvents are approximately the same and E_a-E_e does not appreciably change with the solvents.

It should be pointed out that at first sight the energy difference E_a-E_e may be expected to be about half of that between the (a,a)-form and the (e,e)-form of *trans*-1,4-dichlorocyclohexane[5a], but this is not actually the case.

While the present paper was in preparation, an interesting paper by Klæboe et al.[11], which concerned a part of our investigations, appeared. Their results are in accord with ours.

Appendix

By numbering the carbon atoms of cyclohexane ring from one to six in turn and using the symbols of e and a, we shall represent the potential energies between non-bonded atoms in the molecule of cyclohexane derivative. As for chlorocyclohexane, for instance, the energy between the chlorine atom attached to 1a and the carbon atom of 3 is represented as $V_{1a\,3}$ and that between the same chlorine atom and the hydrogen atom attached to 2e is $V'_{1a\,2e}$.

By the use of these symbols the potential energies, E_a and E_e, of the a- and the e-form of chlorocyclohexane can be represented as follows, respectively;

$$\begin{aligned}E_a=&2V_{1a\,3}+V_{1a\,4}+2V'_{1a\,3a}+2V'_{1a\,3e}+V'_{1a\,4a}\\&+V'_{1a\,4e}+2V'_{1a\,2a}+2U_{1e\,3}+U_{1e\,4}+2U'_{1e\,3a}\\&+2U'_{1e\,3e}+U'_{1e\,4a}+U'_{1e\,4e}+2U'_{1e\,2a}+W \qquad (1)\end{aligned}$$

$$\begin{aligned}E_e=&2V_{1e\,3}+V_{1e\,4}+2V'_{1e\,3a}+2V'_{1e\,3e}+V'_{1e\,4a}\\&+V'_{1e\,4e}+2V'_{1e\,2a}+2U_{1a\,3}+U_{1a\,4}+2U'_{1a\,3a}\\&+2U'_{1a\,3e}+U'_{1a\,4a}+U'_{1a\,4e}+2U'_{1a\,2a}+W \qquad (2)\end{aligned}$$

where V denotes the potential energy between the chlorine and the carbon atom, V' that between the chlorine and the hydrogen atom, U that between the hydrogen and the carbon atom, U' that between the hydrogen atoms attached to the different carbon atoms and W contains all the terms of the potential energies which do not vary by changing the position of the chlorine

9) S. Mizushima, "Structure of Molecules and Internal Rotation", Academic Press Inc., New York, (1954), p. 41.
10) A. Wada, *J. Chem. Phys.*, **22**, 200 (1954).
11) P. Klæboe, J. J. Lothe and K. Lunde, *Acta Chem. Scand.*, **10**, 1465 (1956).

atom from 1a to 1e, and vice versa. As for these equations the equivalent terms are represented by one of them; for instance, as $V_{1e\,3}$ is equal to $V_{1e\,5}$ for Eq. 2, $V_{1e\,3}$ is multiplied by 2.

The potential energies, E_{aa} and E_{ee} of the (a,a)- and the (e,e)-form of *trans*-1,2-dichlorocyclohexane can be similarly represented as follows;

$$\begin{aligned} E_{aa} = {} & 4V_{1a\,3}+2V_{1a\,4}+4V'_{1a\,3a}+4V'_{1a\,3e} \\ & +2V'_{1a\,4a}+2V'_{1a\,4e}+2V'_{1a\,2a}+\Phi_{1a\,2a} \\ & +4U_{1e\,3}+2U_{1e\,4}+3U'_{1e\,2e}+4U'_{1e\,3a} \\ & +4U'_{1e\,3e}+2U'_{1e\,4a}+2U'_{1e\,4e}+\Psi \end{aligned} \tag{3}$$

$$\begin{aligned} E_{ee} = {} & 4V_{1e\,3}+2V_{1e\,4}+4V'_{1e\,3a}+4V'_{1e\,3e} \\ & +2V'_{1e\,4a}+2V'_{1e\,4e}+2V'_{1e\,2e}+\Phi_{1e\,2e} \\ & +4U_{1a\,3}+2U_{1a\,4}+3U'_{1a\,2a}+4U'_{1a\,3a} \\ & +4U'_{1a\,3e}+2U'_{1a\,4a}+2U'_{1a\,4e}+\Psi \end{aligned} \tag{4}$$

where Φ denotes the potential energy between the chlorine atoms attached to the carbon atoms of 1 and 2 and the other symbols have the same meanings as in Eqs. 1 and 2.

By use of Eqs. 1, 2, 3 and 4 and assuming that these terms do not change from molecule to molecule, we can obtain the following equation;

$$(E_{aa}-E_{ee})-2(E_a-E_e)=E_t-E_g.$$

This relation should also be available for the other derivatives in the same way as for the chlorine derivatives.

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*Synthesis of Poly-(L-prolyl-L-leucyl-glycyl). An Attempted Synthesis of Model Collagen**

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(Received March 11, 1958)

The presence of proline residues in a polypeptide chain has two important influences upon its chain configuration. Firstly, the lack of hydrogen atom on the pyrrolidine imide nitrogen makes it impossible to form the hydrogen linkage in the molecule. Secondly, the presence of pyrrolidine rings in the polypeptide chain reduces the degree of freedom of its internal rotation. These effects are supposed to be primarily responsible for the special chain configuration of collagen, gelatin and proline-rich fibrous proteins.

The structural investigations of collagen have been continued by many workers. On the basis of the amino acid analysis[1], it seems to be reasonable that collagen may consist of the polypeptide containing statistically a repeating unit of (glycyl-L-prolyl (or hydroxyprolyl)-X), where X represents any one of amino acid residues other than glycine and proline (or hydroxyproline). Furthermore, the presence of scattering units corresponding to the three amino acid residues in natural collagen was confirmed by X-ray analysis[2]. Following the above considerations, it seems most reasonable to regard the polypeptide with a regular repeating unit of (glycyl-L-prolyl-X) as a simplified model of collagen. Berger et al.[3], prepared poly-L-proline by the *N*-carboxy anhydride method, and the chain configuration of the polypeptide was determined by Cowan and McGavin[4] through X-ray analysis. This polyproline, however, is far from the model collagen.

In principle, the synthesis of high molecular weight polypeptide with a regular repeating unit of two or more amino acid residues is not impossible by the polymerization of tri- or higher membered peptide. For example, tri- or tetrapeptide ester[5], azide[6], thioester[7], mixed anhy-

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1) J. H. Bowes and R. H. Kenten, *Biochem. J.*, **43**, 358 (1948); W. A. Schroeder et al,. *J. Am. Chem. Soc.*, **76**, 3556 (1954).

2) M. L. Huggins, ibid., **76**, 4045 (1954).

3) A. Berger, J. Kurtz and E. Katchalski, ibid., **76**, 5552 (1954).

4) P. M. Cowan and S. McGavin, *Nature*, **176**, 501 (1955).

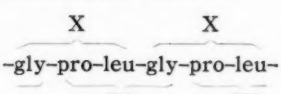
5) E. Pacsu and E. J. Wilson, Jr., *J. Org. Chem.*, **7**, 117, 126 (1942).

6) M. Z. Magee and K. Hofmann, *J. Am. Chem. Soc.*, **71**, 1515 (1949); M. Winitz and J. S. Fruton, ibid., **75**, 3041 (1953).

7) T. Wieland and H. Bernhard, *Ann.*, **582**, 218 (1953).

dride[8], carbothiophenyl derivative[9] and so on are polymerizable into polypeptides and tri- or higher-membered free peptides are polymerizable by the action of some peptide linkage forming reagents such as tetraethyl pyrophosphite[10] and dicyclohexyl carbodiimide[11]. However, these methods contain the hazard of forming ring peptides besides the main product of polypeptides. Then, the optimum condition for high molecular weight polymerization must be investigated in each case where the starting material may be different in solubility and reactivity.

In this paper, the synthesis of poly-(L-prolyl-L-leucyl-glycyl) was attempted in several ways. Firstly, the tripeptide, L-prolyl-L-leucylglycine(IV), and its derivatives were prepared, and the relative efficiencies of polycondensation methods involving dicyclohexyl carbodiimide, tetraethyl pyrophosphite and *p*-nitrothiophenylester methods were compared. Secondly, the relative reactivity for polycondensation was compared between L-prolyl-L-leucylglycine (IV) and glycyl-L-prolyl-L-leucine (X). Although two tripeptides, IV and X, differ in the position of proline residue in the molecule, it must be expected that they should give polypeptides with the same amino acid sequence with the only exception of both *N*- and *C*-terminal amino acid residues.



Synthesis of tripeptides and their derivatives. — Carbobenzoxy-L-prolyl-L-leucylglycine (III) was prepared according to the description of Ressler and du Vigneaud[12], and the protecting group was removed by hydrogenation in the presence of palladium-charcoal.

The tripeptide thioester, L-prolyl-L-leucylglycine *p*-nitrophenylthioester hydrobromide, was prepared by the procedure of Schwyzer[13]. Such a peptide *p*-nitrophenyl thioester was used for the preparation of the cyclic peptide by kenner[14].

The synthesis of ethyl carbobenzoxyglycyl-L-prolyl-L-leucinate (VIII) was accomplished by coupling carbobenzoxyglycyl-L-proline with ethyl L-leucinate through both mixed anhydride[15] and carbodiimide procedures[16]. After saponification of the product, the carbobenzoxy tripeptide (IX) was hydrogenated into glycyl-L-prolyl-L-leucine (X) similarly. On the formation of the peptide linkage, dicyclohexyl carbodiimide gave generally good results, but in the course of saponification of ethyl carbobenzoxyglycyl-L-prolyl-L-leucinate (VIII), a small amount of nonsaponifiable substance remained. This material may be the addition product of carbodiimide with carbobenzoxy peptide, as was reported by other workers[16,17].

Carbobenzoxyglycyl-L-prolyl-L-leucine (IX) was hard to dissolve in most usual organic solvents and exhibited a marked contrast to the great solubility of carbobenzoxy-L-prolyl-L-leucylglycine (III). Both the free tripeptides, L-prolyl-L-leucylglycine (IV) and glycyl-L-prolyl-L-leucine (X), were easily soluble in glacial acetic acid and water, sparingly soluble in hot methanol containing some water and in hot dimethylformamide, and insoluble in other usual organic solvents. These solubility behaviors rather limited the experimental conditions for polycondensation. The tripeptide, glycyl-L-prolyl-L-leucine (X), contained one molecule of firmly bound crystallization water, and it could not be removed by heating over phosphorus pentoxide at 135°C(2 mmHg) for six hours. The significance of this water will be discussed in a later section.

Polycondensation of tripeptides. — In the polycondensation of L-prolyl-L-leucylglycine (IV) by the carbodiimide and the tetraethyl pyrophosphite procedures or in the polycondensation of its *p*-nitrothiophenylester, the most promising result was obtained by pyrophosphite. The most favorable molar ratio of this reagent to the tripeptide was about 1.5~2, the amount of consumption of the reagent by inevitable contamination of water being taken into consideration, and excess or insufficiency of reagent reduced the molecular

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11) V. Bruckner, M. Szekerke and J. Kovács, *Naturwiss.*, **43**, 107 (1956).
12) C. Ressler and V. du Vigneaud, *J. Am. Chem. Soc.*, **76**, 3107 (1954).
13) R. Schwyzer, B. Iselin, W. Rittel and P. Sieber, *Helv. Chim. Acta*, **39**, 872 (1956).
14) G. W. Kenner and J. M. Turner, *Chem. and Ind.*, **1955**, 602.
15) J. R. Vaughan, Jr. and R. L. Osato, *J. Am. Chem. Soc.*, **73**, 5553 (1951).
16) J. C. Sheehan and G. P. Hess, ibid., **77**, 1067 (1955); ibid., **78**, 1367 (1956).
17) H. Zahn and J. F. Diehl, *Angew. Chem.*, **69**, 135 (1957).

weight of polypeptide formed. Tetraethyl pyrophosphite is very sensitive to moisture and the estimation of its purity was difficult, hence, the reproducibility of the result was rather inadequate.

The use of carbodiimide for polycondensation of L-prolyl-L-leucylglycine (IV) was limited by the low solubility of the tripeptide in inert solvents such as dimethylformamide at room temperature. At higher temperature, although the tripeptide dissolved in dimethylformamide, none of the free carboxyl group was detected in the product, where the addition reaction of the reagent to the end-carboxyl group may proceed in the course of polycondensation.

The *p*-nitrophenyl thioester procedure for polycondensation was also limited by the lack of available solvent, quite different from the method of Kenner[14] for cyclic peptides. In this reaction, it was considered to be reasonable that anhydrous organic solvents were preferred to water, because water would not be expected to dissolve the high molecular-weight polypeptide. Equivalent triethylamine was used for the separation of hydrogen bromide, but the reacting solution became colored markedly and the difficulty of separation of sulfur-containing compound from the product gave an undesirable result.

The polycondensation reaction by the pyrophosphite procedure was carried out at 100°, so it was supposed that the thermal side reaction might occur in the process and that equilibrium might exist between peptide linkages of the polycondensate. If the peptide-bond exchange or chain degradation occurs to any appreciable degree, the sequence of amino acid residues in the polypeptide chain would no longer retain that of the starting tripeptide. In order to clarify these questions, the nature of end amino acid residues and the content of phosphorus were determined on the products from the tripeptide(IV). After the complete hydrolysis of the polypeptide, proline, leucine and glycine were determined by paper chromatography. No appreciable amount of phosphorus was detected. From the amino nitrogen determination by the manometric van Slyke method, the free NH_2 group could not be detected either, so the *N*-terminal residue was assumed to be wholly L-proline. Determination of *C*-terminal amino acid residue of this polypeptide by hydrazinolysis[18] gave only DNP-glycine. All of these results indicated that no change in the sequence of amino acid residues has occurred during the course of the polycondensation by tetraethyl pyrophosphite.

The position of prolyl residue in tripeptide should have some influences on the peptide chain configuration which would affect the reactivity of either or both end groups in the polycondensation process. This factor must also depend upon the nature of end amino acid residues. To see these effects, the polycondensation reactions of both the tripeptides, L-prolyl-L-leucylglycine (IV) and glycyl-L-prolyl-L-leucine (X), were compared in parallel runs under the same conditions by the tetraethyl pyrophosphite procedure. The comparison indicated that the former gave a higher molecular weight product. This result differs from the expectation that the *N*-terminal glycyl residue on the tripeptide (IV) would be more reactive than the *N*-terminal prolyl residue on the tripeptide (X). Taking into consideration the results of Rydon[19], the latter peptide may be oriented in a rather rigid chain configuration.

Poly-(glycyl-L-prolyl-L-leucyl), prepared from both the tripeptides, IV and X, contained very firmly bound water, the amount of which was one mole per tripeptide unit. The tripeptide, glycyl-L-prolyl-L-leucine (X), also contained one molecule of crystallization water, as mentioned above. In addition, many other workers[19,20] have also reported the presence of crystallization water in the oligopeptides containing glycyl-prolyl residues. These facts suggest that the glycyl-prolyl linkage always has an ability to fix water molecule very firmly. If this hydration ability is general, this factor also may play a major role in the configuration of polypeptide chain of gelatin and collagen in addition to the other effects described in the first section.

The highest molecular weight fraction of the products by the pyrophosphite procedure had a molecular weight of about 6000, and this material was rather brittle and all efforts to make it into film or fiber form failed. This material showed three peaks (12.0; 6.6; 4.0 Å) in the X-ray diffraction chart by using Norelco diffractometer.

18) S. Akabori et al., This Bulletin, **29**, 507 (1956).
19) H. N. Rydon and P. W. G. Smith, *J. Chem. Soc.*, **1956**, 3642.
20) J. R. Vaughan and J. A. Eichler, *J. Am. Chem. Soc.*, **76**, 2474 (1954); N. C. Davis and E. L. Smith, *J. Biol. Chem.*, **200**, 373 (1953).

Experimental

Ethyl carbobenzoxy-L-leucylglycinate (I).—To a solution of 2.65 g. of carbobenzoxy-L-leucine[21] and 2.3 g. of dicyclohexyl-carbodiimide[22] in 20 ml. of tetrahydrofuran was added a solution of 1.67 g. of ethyl glycinate hydrochloride and 1.25 g. of triethyl amine in 10 ml. of tetrahydrofuran. The mixture was allowed to stand at room temperature for 4 hours. A small amount of acetic acid was added to the reaction mixture and after 30 minutes, the separated urea derivative was filtered off. The mother liquor was evaporated to dryness under reduced pressure and the residue was dissolved in ethyl acetate. The solution was washed with three portions of sodium bicarbonate solution, one portion of 0.2 N hydrochloric acid and water successively, and dried over magnesium sulfate. Evaporation of the solvent gave a viscous syrup, from which crystals separated out immediately on the addition of some petroleum ether. The colorless needles obtained were recrystallized from aqueous ethanol; m.p. 102~103°C and $[\alpha]_D^{18}$ −26.94° (c 5.01, ethanol). The yield was 2.65 g. (75%). Anderson et al.[23] reported a yield of 80%, m.p. 104~105°C and $[\alpha]_D^{25}$ −26.4°, and also yield of 65%, m.p. 102~103°C and $[\alpha]_D^{25}$ −27.2°. Bergmann[24] reported m. p. 103~104°C.

Ethyl carbobenzoxy-L-prolyl-L-leucylglycinate (II).—This substance was prepared from carbobenzoxy-L-proline[3] and I according to the description of du Vigneaud et al.[12] by the mixed anhydride procedure using isovaleryl chloride in 92% yield; m. p. 147~148°C, $[\alpha]_D^{18}$ −80° (c 2.5, ethanol). These values were identical with those reported previously[12].

Carbobenzoxy-L-prolyl-L-leucylglycine (III).—This substance was prepared from II according to the description of du Vigneaud et al.[12] in 91.5% yield; m. p. 163~164°C, $[\alpha]_D^{22.5}$ −85.0° (c 3.23, ethanol). These values were identical with those reported previously[12].

L-Prolyl-L-leucylglycine (IV).—To a solution of 6 g. of III in 150 ml. of methanol containing a small amount of glacial acetic acid was added 4 g. of palladium-charcoal, and hydrogen was introduced into the suspension for three hours under stirring. The catalyst was filtered off and washed thoroughly with distilled water, and then the combined filtrate was concentrated under reduced pressure. The residual syrup was dried by the addition of benzene followed by evaporation under reduced pressure. The remaining white powdery mass was dissolved in 10% methanolic acetic acid and reprecipitated with hot ethyl acetate. The yield of the colorless needles which were obtained was 3.5g. (84.5%); m.p. 220°C (decomp.), $[\alpha]_D^{20}$ −83.7° (c 3.43, glacial acetic acid).

Anal. Found: C, 54.54; H, 8.01; N, 14.80; mol. wt. 290*. Calcd. for $C_{13}H_{23}O_4N_3$: C, 54.75; H, 8.13; N, 14.73%; mol. wt. 285.3.

Paper chromatography showed this tripeptide to have only one spot, which turned yellow with ninhydrin, with an R_f value of 0.74 in *n*-butanol: water: acetic acid (4:1:1). The *p*K' values, 4.0 and 9.5, of the tripeptide were determined by the method of Parke and Davis[25].

Carbobenzoxy-L-prolyl-L-leucylglycine *p*-nitrophenyl-thioester (V).—A solution of 1.85 g. of III in 5 ml. of dimethylformamide was mixed with a solution of 0.45 g. of triethylamine in 5 ml. of ethylacetate and the mixture was cooled to −5°C. A solution of 0.48 g. of ethyl chlorocarbonate in ethyl acetate was then added dropwise into the reaction mixture at −5°C under stirring. After ten minutes a solution of 0.69 g. of *p*-nitrothiophenol and 0.45 g. of triethylamine in 10 ml. of ethyl acetate was added into the reaction mixture, which was allowed to stand for one hour at room temperature, when the dark red color of the mixture vanished gradually. The reacted solution was washed with dilute hydrochloric acid and dried over magnesium sulfate. Evaporation of the solvent followed by addition of petroleum ether, gave colorless crystals. The yield was 2.22 g. (90%). After recrystallization from aqueous ethanol, this substance melted at 137~138.5°C; wt. 1.9 g.

Anal. Found: C, 58.13; H, 5.99; N, 9.99. Calcd. for $C_{27}H_{32}O_7N_4S$: C, 58.25; H, 5.75; N, 10.08%.

L-Prolyl-L-leucylglycine *p*-nitrophenyl-thioester hydrobromide (VI).—A solution of 1.75 g. of V in 5 ml. of glacial acetic acid was saturated with dry hydrogen bromide. After one hour at room temperature, the solution was concentrated under reduced pressure and the residual syrupy mass was reprecipitated from glacial acetic acid by the addition of dry ether. The yield of the white powder was 1.57 g. (97%); m. p. 170°C (decomp.). This material was very hygroscopic and its aqueous alkaline solution was dark red. Analytical value of bromine determined by the Volhard method was 94% of the theoretical, but further purification of the substance was unsuccessful.

Carbobenzoxyglycyl-L-proline (VII).—This substance was prepared from carbobenzoxyglycyl chloride and L-proline according to the description of Bergmann et al.[26] in 77% yield; m. p. 155°C. Rydon[19] reported m.p. 155°C and Bergmann reported m. p. 156°C.

Ethyl carbobenzoxyglycyl-L-prolyl-L-leucinate (VIII).—This substance was prepared both

21) M. Bergmann, L. Zervas and J. S. Fruton, *J. Biol. Chem.*, **115**, 593 (1936).
22) E. Schmidt, F. Hitzler and E. Lahde, *Ber.*, **71**, 1933 (1938).
23) G. W. Anderson et al., *J. Am. Chem. Soc.*, **74**, 5307, 5309 (1952).
24) M. Bergmann, L. Zervas and J. S. Fruton, *J. Biol. Chem.*, **111**, 225 (1935).

* The molecular weight was determined by the end-carboxyl titration using sodium methoxide in dimethylformamide: J. S. Fritz and N. M. Lisicki, *Anal. Chem.*, **23**, 589 (1951); M. Sela and A. Berger, *J. Am. Chem. Soc.*, **77**, 1893 (1955).
25) T. V. Parke and W. W. Davis, *Anal. Chem.*, **26**, 642 (1954).
26) M. Bergmann et al., *Ber.*, **65**, 1192 (1932); *Z. physiol. Chem.*, **212**, 72 (1932).

by carbodiimide and by mixed anhydride procedures, but all efforts for crystallization failed in every case. (a) Carbodiimide procedure. To a solution of 9.3 g. of VII and 6.8 g. of dicyclohexyl-carbodiimide in 70 ml, of tetrahydrofuran was added a suspension of 7.5 g. of ethyl L-leucinate hydrochloride and 3.82 g. of triethylamine in 50 ml. of tetrahydrofuran, and the mixture was allowed to stand overnight. The reacted mixture was treated in the same manner as in the case of I and 13 g. of syrupy mass was obtained. (b) Mixed anhydride procedure. Two grams of VII, 0.7 g. of isovaleryl chloride and 1.3 g. of ethyl L-leucinate were treated in the same manner as in the case of II in tetrahydrofuran and 2.3 g. of the syrupy mass was obtained.

Carbobenzoxyglycyl-L-prolyl-L-leucine (IX).—To a solution of 13 g. of VIIIa in 40 ml. of acetone was added 16 ml. (1.1 equivalent) of 2 N sodium hydroxide. After 2.5 hours at room temperature (10°C) the solution was acidified and the separated oil was extracted with ethyl acetate. The ethyl acetate solution was extracted again with aqueous sodium bicarbonate, and the aqueous layer was acidified. A white crystalline solid separated, which was filtered off. After recrystallization from a large amount of aqueous methanol this solid gave 8.4 g. (66% based on VII) of the colorless plates; m. p. 201~202°C, $[\alpha]_D^{13}$ −62.7° (c 1.4, dimethyl formamide).

Anal. Found: C, 60.51; H, 6.75; N, 10.08. Calcd. for $C_{21}H_{29}O_6N_3$: C, 60.13; H, 6.97; N, 10.02%.

Glycyl-L-prolyl-L-leucine (X).—To a solution of 2.7 g. of IX in 100 ml. of methanol containing a small amount of glacial acetic acid was added 2 g. of palladium-charcoal, and hydrogen was introduced into the suspension for two hours under stirring. Then, the catalyst was filtered off and washed with water. The combined filtrate was concentrated to dryness under reduced pressure, and colorless needles of 1.55 g. were obtained in 85% yield. After recrystallization from aqueous methanol, the substance decomposed at 180~190°C; $[\alpha]_D^{9.5}$ −124° (c. 2.16, glacial acetic acid), $[\alpha]_D^{20.5}$ −125.1° (c 3.1, water).

Anal. Found*: C, 52.43; H, 8.15; N, 13.53. Calcd. for $C_{13}H_{23}O_4N_3 \cdot H_2O$: C, 51.47; H, 8.37; N, 13.85%. Found**: C, 54.61; H, 7.18; N, 15.00. Calcd. for $C_{13}H_{23}O_4N_3$: C, 54.75; H, 8.13; N, 14.73%. Paper chromatography showed this tripeptide to have only one spot, which turned yellow with ninhydrin, with an Rf value of 0.72 in *n*-butanol: water: acetic acid (4 : 1 : 1).

Polycondensation of L-prolyl-L-leucylglycine by the pyrophosphite procedure.—The suspension of 2.74 g. of IV and 4.0 g. of tetraethylpyrophosphite (1.5 equivalent against IV) in 7 ml. of diethylphosphite was sealed in a glass tube and heated in a boiling water bath. After about 5 minutes the tripeptide dissolved clearly in the solvent, and after about half an hour another slight precipitate appeared. The heating was continued for another hour, but no increase was observed in the amount of the precipitate. After cooling to room temperature 2 ml. of water was added into the reaction mixture in order to hydrolyse the excess reagent and active centers in the product. Removal of the diethylphosphite and water under reduced pressure left the polycondensation product as a white amorphous solid, which was treated with ethanol and ether for pulverization. This product was soluble in hot ethanol and partially soluble in hot methanol. After the hot methanol-soluble part was extracted out, the residue was dissolved in hot ethanol and fractionated with ether into three parts; namely, the first precipitate (Fr-1), the second precipitate (Fr-2) from the first mother liquor, and the residual part (Fr-3) which was obtained from the second mother liquor after removal of the solvent and the treatment with ether. The hot methanol extract was concentrated to dryness and the residue was pulverized by adding ether (Fr-4). The first fraction (Fr-1) was further fractionated in two parts, (Fr-1A) and (Fr-1B), using dimethylformamide and ether. The yields and the molecular weights of these fractions are given in Table I.

TABLE I

YIELD OF EACH FRACTION AND ITS MOLECULAR WEIGHT OF THE POLYCONDENSATE OF L-PROLYL-L-LEUCYLGLYCINE BY THE PYROPHOSPHITE PROCEDURE

Fraction number	Yield (mg.)	Mol. wt.*
Fr-1	480	—
Fr-1A	90	6600
Fr-1B	340	4200
Fr-2	450	3000
Fr-3	440	2000
Fr-4	615	1200
Total	1,985 mg. (77% based on IV)	

* These values were determined by the end-carboxyl titration using sodium methoxide in dimethylformamide: M. Sela and A. Berger, *J. Am. Chem. Soc.*, **77**, 1893 (1955).

The polypeptide (Fr-1B) was soluble in glacial acetic acid, pyridine, dimethylformamide and hot ethanol, and insoluble in water, benzene, chloroform and ethyl acetate; m. p. 190°C (decomp), $[\alpha]_D^{20}$ −104° (c 5.3, dimethylformamide). This material showed no biuret reaction.

Anal. Found: C, 54.81; H, 7.82; N, 14.70. Calcd. for $(C_{13}H_{21}O_3N_3 \cdot H_2O)_n$: C, 54.75; H, 8.13; N, 14.73%.

The phosphorus content of this material was determined by the method of Fiske[27], but it was only less than 0.01%. No amino nitrogen was detected by the manometric van Slyke method. This material was hydrazinolysed[18] with

* This substance was dried at 118°C (2 mm.) for 6 hours.

** This substance was dried at 138°C (2 mm.) for 6 hours. In this state it was strongly hygroscopic and easily returned to the initial state by being carelessly allowed to stand in the air.

27) C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, **66**, 375 (1925).

anhydrous hydrazine for 12 hours and the reaction mixture was dinitrophenylated. Thus, only one spot corresponding to DNP-glycine was detected by paper chromatography[28] from the acidic part of the reaction mixture, in which all the *C*-terminal amino acid of the polypeptide must be included.

The lower molecular weight fractions (Fr-3, Fr-4) were treated once more with tetraethylpyrophosphite in the same manner as described above, but no molecular weight increase was observed.

Polycondensation of L-prolyl-L-leucylglycine by the carbodiimide procedure.—One gram of dicyclohexylcarbodiimide and 1.07 g. of IV were suspended in 5 ml. of dimethylformamide and the suspension was sealed in a glass tube, but no change in the appearance of this suspension was observed at room temperature. When the mixture was heated at 70°, it changed to a clear solution. After 3 hours the solution was cooled to room temperature and the dimethylformamide was removed under reduced pressure. The residual syrup was treated with ethanol and ether, and 1.0 g. of crystalline product was obtained. However, the separation of polypeptide from the product was unsuccessful and no free carboxyl group was detected in this material by the sodium methoxide titration.

Polycondensation of L-prolyl-L-leucylglycine *p*-nitrophenylthioester.—The solution of 500 mg. of VI and an equivalent of triethylamine in 2 ml. of dimethylformamide was sealed in a glass tube and heated at 60° for 15 minutes. After standing at room temperature for 18 hours the resulting dark red solution changed to light yellow on the addition of a small amount of hydrochloric acid, and then excess of water was added to the solution. The precipitate formed was filtered off and dissolved in glacial acetic acid. Some hydrogen peroxide was added to the solution and the crystals which separated were filtered off. The mother liquor was concentrated under reduced pressure, and then the residual mass was extracted with hot ethanol. The extract was concentrated to dryness and the residue was treated twice in the same way as described above. The last amorphous residue was purified with dimethylformamide and ether, and about 100 mg. of white powdery substance was obtained. The molecular weight of this fraction (1150) was determined by end-carboxyl titration, but no analytical data were determined.

Parallel runs of the polycondensation reaction of L-prolyl-L-leucylglycine (IV) and of glycyl-L-prolyl-L-leucine (X).—The tripeptides IV and anhydrous X were treated in parallel runs under the same condition by the pyrophosphite procedure. The results are listed in Table II.

TABLE II

COMPARISON OF THE POLYCONDENSATION PRODUCTS OF THE TWO TRIPEPTIDES IV AND X

Tripeptide	mg.	Diethylphosphite ml.	Tetraethylpyrophosphite mg.	Yield mg.	Yield %	Mol. wt.
IV	400	3.0	700	250	60	3000
X	400	3.0	700	130	35	1900
IV	400	2.5	1300	300	80	550
X	400	2.5	1300	170	45	500

Summary

The two tripeptides, L-prolyl-L-leucylglycine (IV) and glycyl-L-prolyl-L-leucine (X), were synthesized, and IV was subjected to polymerization by the tetraethyl pyrophosphite, carbodiimide and *p*-nitrophenyl thioester methods, in which the most promising result was obtained by the pyrophosphite method. The highest molecular weight of the polypeptide which was obtained was about 6000 and the repetition of the original tripeptide unit in the polypeptide was confirmed by the end groups determination. The polycondensation reactions of both the tripeptides, IV and X, were compared in parallel runs by the tetraethyl pyrophosphite procedure, and the comparison showed that IV gave a better yield and a higher molecular weight product than X. X and poly-(L-prolyl-L-leucyl-glycyl), which was obtained from IV, contained firmly bound water in spite of the fact that IV contained no crystallization water.

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28) A. L. Levy, *Nature*, **174**, 126 (1954).

Synthetic Amphoteric Polypeptide. III. A Water-Soluble Amphoteric Polypeptide

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In the preceding paper[1] it was reported that the synthetic polypeptides, copoly-1:1:3-(DL-glutamic acid, DL-lysine, DL-phenylalanine) and copoly-1:1:1.9-(L-glutamic acid, L-lysine, L-leucine), are insoluble in water in the neutral pH region. On the other hand, in view of the great solubility of polyserine[2] in water, it may be considered that the water-solubility of a number of proteins, such as albumins, should be contributed largely by the serine and threonine residues in these proteins. To confirm this, it is desired to obtain an amphoteric polypeptide which contains any amount of serine residue as a component and is soluble in water over a whole pH range. In order to know how many serine residues are required to make the polypeptide freely soluble in water, four copolymers composed of various compositions of L-glutamic acid and DL-serine were synthesized and their solubilities were tested. While α-poly-L-glutamic acid is sparingly soluble in water in its free acid form, it was found that the copoly-(L-glutamic acid, DL-serine) was freely soluble in water, if the content of serine residue becomes more than about 50 mole per cent; hence the synthesis of copoly-1:1:2-(L-glutamic acid, L-lysine, DL-serine), as a water-soluble amphoteric polypeptide, was investigated.

The four copolymers composed of L-glutamic acid and DL-serine, the molar ratios of which were 4:1, 3:2, 2:3 and 1:4, and copoly-1:1:2-(L-glutamic acid, L-lysine, DL-serine) were obtained from four corresponding copoly-(γ-benzyl L-glutamate, *O*-benzyl-DL-serine) and copoly-1:1:2-(γ-benzyl L-glutamate, ε-*N*-carbobenzoxy-L-lysine, *O*-benzyl-DL-serine), respectively, which were prepared by the *N*-carboxy anhydride method as described in the preceding paper[1]. In the debenzylation reaction of these copolymers some attention should be paid to the occurrence of side reaction, because in most cases some degree of ester linkages may be formed during the reaction between hydroxyl group of serine residue and carboxyl group of either glutamic acid residue or of an acid used as solvent.

The debenzylation of copoly-(γ-benzyl L-glutamate, *O*-benzyl-DL-serine) was carried out in dioxane with dry hydrogen bromide, and in acetic acid with phosphonium iodide. The reaction with hydrogen bromide in dioxane gave the products in theoretical amount all insoluble, but swellable, even in hot aqueous potassium hydrogen carbonate. Poly-L-glutamic acid and poly-DL-serine, prepared under similar conditions from poly-γ-benzyl-L-glutamate and poly-*O*-benzyl-DL-serine, were readily soluble in aqueous potassium hydrogen carbonate and in water respectively. Those insoluble products obtained from the copolymers could be converted into soluble forms by treating with aqueous triethylamine (pH 10) at room temperature overnight. Under this condition, no change in the content of amino-nitrogen of poly-DL-serine and in the value of specific rotation of poly-L-glutamic acid was observed. These results indicate clearly that the inter- and/or intra-chain ester linkages were broken by a fairly mild condition, such as aqueous triethylamine, without accompanying any hydrolysis of the peptide bond or racemization.

The reaction of phosphonium iodide on the four copolymers in glacial acetic acid gave the products, the yields of which somewhat exceeded the theoretical amounts, partly insoluble in aqueous potassium hydrogen carbonate. These products could also be converted into perfectly soluble forms by treating with aqueous triethylamine, and may be considered as polymers the hydroxyl groups of which had been partly esterified both by acetic acid used as solvent and by the carboxyl groups of glutamic acid residues.

The final products obtained by these

1) H. Yuki, S. Sakakibara and H. Tani, This Bulletin, **29**, 654 (1956).

2) K. Okawa and H. Tani, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **75**, 1199 (1954).

two different processes were all soluble in aqueous potassium hydrogen carbonate and copoly-2:3- and copoly-1:4-(L-glutamic acid, DL-serine) were soluble even in water. When dried in vacuo at an elevated temperature, these polypeptides showed a slight tendency to become insoluble in aqueous potassium hydrogen carbonate, but could readily be returned soluble by heating in this medium.

It was found that a pyrrolidone ring may be formed by the ring-closure of glutamyl residue, existing at the amino-end of these polymer molecules, because no free amino-nitrogen was detected in poly-L-glutamic acid prepared by this method.

The optical rotations of these copoly-(L-glutamic acid, DL-serine) were measured in their anionic forms and the intrinsic rotation of L-glutamic acid residue was calculated for each polypeptide, as the serine residues might be regarded to have no contribution to the optical rotation of the polymer since they were racemic. These values were almost constant ($-50°$ to $-57°$) and differed from that of poly-L-glutamic acid ($-88°$).

When the debenzylation of copoly-1:1:2-(γ-benzyl L-glutamate, ε-*N*-carbobenzoxy-L-lysine, *O*-benzyl-DL-serine) was carried out in glacial acetic acid with phosphonium iodide, it gave a cross-linked product insoluble, but swellable, in various aqueous and organic media, and this product could not be turned soluble by treating with triethylamine. The debenzylation of this copolymer was effected in dichloroacetic acid by using the theoretical amount of phosphonium iodide satisfactorily without formation of any cross-linkage. From the resulting polypeptide hydriodide, free copoly-1:1:2-(L-glutamic acid, L-lysine, DL-serine) was obtained by treating repeatedly with triethylamine.

This amphoteric polypeptide was found to be soluble in water over the whole pH range. Intrinsic viscosity of its solution in water was 0.04, and such small $[\eta]$ value may reflect rather low molecular weight. In view of the known lability of serine peptide to acid, the molecular weight depression, if any, might be due to the action of hydrogen iodide during the debenzylation process.

Dependence of specific viscosity on pH is shown in Fig. 1. The viscosity was minimum at the isoelectric point (pH 7), and increased gradually on both sides. This behavior is typical of amphoteric polyelectrolyte.

Dependence of specific rotation on pH was shown in Fig. 2. The specific rotation was *laevo*-rotatory over whole pH range; the value was constant ($-32°$) from pH 3.6 to pH 11.3, and increased rapidly on both sides. This specific rotation *vs* pH curve is very similar to that of serum albumin[3] and of ovalbumin[4].

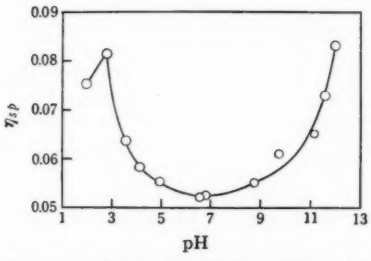

Fig. 1. Relation between specific viscosity of copoly-1:1:2-(L-glutamic acid, L-lysine, DL-serine) and pH.

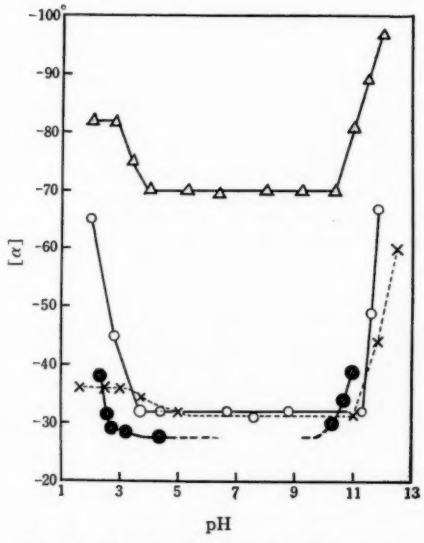

Fig. 2. Relation between specific rotation and pH.
○, Copoly-1:1:2-(L-glutamic acid, L-lysine, DL-serine); ●, Copoly-1:1:1.9-(L-glutamic acid, L-lysine, L-leucine)[D]; △, Serum albumin[3]; ×, Ovalbumin[4].

The optical rotatory behavior of synthetic polypeptide was discussed recently

3) M. A. Colub and E. E. Pickett, *J. Polymer Sci.*, **13**, 427 (1954).
4) J. T. Edsall and J. F. Foster, *J. Am. Chem. Soc.*, **70**, 1860 (1948).

both theoretically[5] and experimentally[6-8], and the workers concerned concluded that the chain configuration of the polymer contributes a great deal to this behavior. In the polypeptides obtained here, serine residue is DL-form and hence the problem of chain configuration would be rather complex[9], but it is very interesting that the behavior concerning optical rotation of this synthetic amphoteric polypeptide is quite similar to that of albumins; their specific rotations are *laevo*-rotatory in the whole pH range and have minimum constant values over wide ranges of pH around their isoelectric points. This behavior may correspond to the observation of P. Doty and his collaborators[8], who found that the specific rotation of poly-L-glutamic acid is constant until about 40% of the side-chain carboxyl group are dissociated. The reason for the very interesting result that the intrinsic rotation of glutamic acid residue in copoly-(L-glutamic acid, DL-serine) is almost constant in spite of the great difference of glutamic acid content and differs from that of poly-L-glutamic acid, remained obscure.

Although the viscosity and optical rotation could be measured on copoly-1 : 1 : 1.9-(L-glutamic acid, L-lysine, L-leucine)[1] only at both sides of the neutral pH region, because of its water-insolubility in the neutral pH region, the object of the measurements of these properties could now be attained completely on copoly-1 : 1 : 2-(L-glutamic acid, L-lysine, DL-serine) over the whole pH range. Some physicochemical properties of these synthetic amphoteric polypeptides are very similar to those of some natural proteins, and the latter, copoly-1 : 1 : 2-L-glutamic acid, L-lysine, DL-serine), may be regarded as the first member of 'model albumin', if the former, copoly-1 : 1 : 1.9-(L-glutamic acid, L-lysine, L-leucine)[1], together with copoly-1 : 1 : 3-(DL-glutamic acid, DL-lysine, DL-phenylalanine)[1], might be called 'model globulin'.

Experimental

Poly-γ-benzyl L-glutamate, poly-*O*-benzyl-DL-serine, and copoly-(γ-benzyl L-glutamate, *O*-benzyl-DL-serine).—Six different mixtures of *N*-carboxy-γ-benzyl L-glutamate anhydride[1] and *N*-carboxy-*O*-benzyl-DL-serine anhydride[2] were prepared: their molar ratios were 1:0, 4:1, 3:2, 2:3, 1:4 and 0:1, and the total amount was 10 m mol. in each mixture. Ten percent solution of each mixture in chlorobenzene was heated in a sealed glass tube for 2 hours at 90°C and then for one week at 40°C. The polymer mas precipitated by adding petroleum ether, washed with methanol and ether and dried. The results are shown in Table I.

Polymer G was soluble in dioxane, tetrahydrofurane, chloroform, nitrobenzene, pyridine and dichloroacetic acid, and was insoluble in water, ethanol and glacial acetic acid. While the solubility of polymer I was similar to that of polymer G polymer, II was soluble only in dichloroacetic acid and in hot dioxane, and polymer III, IV and S were soluble only in dichloroacetic acid.

TABLE I

Polymer	Molar ratio A/B	Appearance of polymerizing solution	Yield %		Anal. % C	H	N	Formula*
G	1 : 0	viscous solution	97	Calcd.			6.5	$[A]_n$
				Found			6.4	
I	4 : 1	viscous solution	97	Calcd.	66.1	6.0	6.6	$[4A, B]_n$
				Found	65.7	6.4	6.6	
II	3 : 2	transparent gel	93	Calcd.	66.4	6.1	6.9	$[3A, 2B]_n$
				Found	65.9	6.4	7.0	
III	2 : 3	opalescent gel	94	Calcd.	66.8	6.1	7.2	$[2A, 3B]_n$
				Found	65.8	6.2	7.2	
IV	1 : 4	opalescent gel	92	Calcd.	67.3	6.2	7.6	$[A, 4B]_n$
				Found	66.4	6.2	7.6	
S	0 : 1	opalescent gel	91	Calcd.			7.9	$[B]_n$
				Found			8.0	

*A: γ-Benzyl L-glutamate residue, $C_{12}H_{13}NO_3$.
B: *O*-Benzyl-DL-serine residue, $C_{10}H_{11}NO_2$.

5) D. D. Fitts and J. G. Kirkwood, ibid., **78**, 2650 (1950); *Proc. Natl. Acad. Sci.*, **42**, 33 (1956).
6) E. R. Blout and M. Idelson, *J. Am. Chem. Soc.*, **78**, 497 (1956).
7) P. Doty and J. T. Yang, ibid., **78**, 500 (1956).
8) P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Polymer Sci.*, **23**, 851 (1957).
9) A. Elliott, W. R. Hanby and B. R. Malcolm, *Nature*, **178**, 1170 (1956); E. R. Blout, P. Doty and J. T. Yang, *J. Am. Chem. Soc.*, **79**, 759 (1957).

TABLE II

Product	Reaction condition: Polymer/Dioxane mg./ml.	Reaction time* min.	Yield** %		Anal.** % C	H	N	Formula***
G′	500/15	60	101	Found			10.5	$[A']_n$
				Calcd.			10.9	
I′	400/15	25	100	Found	44.5	5.9	11.3	$[4A', B']_n$
				Calcd.	45.8	5.5	11.6	
II′	180/12	20	106	Found	41.3	5.8	11.8	$[3A', 2B']_n$
				Calcd.	44.9	5.6	12.5	
III′	300/13	15	100	Found	41.2	6.0	12.7	$[2A', 3B']_n$
				Calcd.	43.9	5.6	13.5	
IV′	400/15	25	101	Found	40.0	5.5	13.7	$[A', 4B']_n$
				Calcd.	42.8	5.7	14.7	
S′	400/10	20	94	Found			15.2	$[B']_n$
				Calcd.			16.1	

* The time hydrogen bromide passed through; during this period the polymer was completely precipitated.

** Each sample was dried over phosphorus pentoxide at 108°C/2 mmHg for five hours, but the data show that even after this treatment, the polymer may contain about 3~6% of water.

*** A′: Glutamic acid residue, $C_5H_7O_3N$. B′: Serine residue, $C_3H_5O_2N$.

TABLE III

Polypeptide	Intrinsic rotation of glutamic acid residue $[\alpha_{glu}]_D$	Insoluble pH range	Amino-nitrogen %
G′	−88°*(at pH 8.3, 10.4)	below 3.9	0.00
I′	−50° (at pH 9.6)	below 3.5	0.04
II′	−56° (at pH 9.8)	below 1.1	0.18
III′	−57° (at pH 9.8)	none	0.21
IV′	−51° (at pH 10.1)	none	0.18
S′	0°	none	0.19 (at pH 6 and 10.1)

* Reported value[6]: −83°.

Poly-L-glutamic acid, poly-DL-serine and copoly-(L-glutamic acid, DL-serine).—(*a*) *Debenzylation with hydrogen bromide in dioxane.* To a mixture of the polymer and dioxane, dry hydrogen bromide was passed at room temperature, the solvent was distilled off in vacuo, and the residue was washed successively with anhydrous ether, methanol, and ether. After drying, white powder was obtained. The results are shown in Table II.

While polypeptide G′ was soluble in aqueous potassium hydrogen carbonate and polypeptide S′ was soluble in water, the others were insoluble but swellable in aqueous potassium hydrogen carbonate. A suspension of each insoluble copolymer in water containing an equivalent amount of potassium hydrogen carbonate was changed to a clear solution by adding a few drops of triethylamine and on standing overnight at room temperature. The optical rotation, pH and amino-nitrogen value were determined on each solution. No change in these values was observed even after standing the solution for two weeks. In Table III the value of $[\alpha_{glu}]_D$, the intrinsic rotation of L-glutamic acid residue, was calculated based on the cencentration of glutamic acid residue contained in the solution, but not on the concentration of polymer itself.

(*b*) *Debenzylation with phosphonium iodide in glacial acetic acid.* — About 1.4 g. of weighed polymer sample (I, II, III or IV) was suspended in 75 ml. of glacial acetic acid. To this mixture 4~5 g. phosphonium iodide was added in portions at 65~65°C during 4.5 hours with passing through dry hydrogen. The solvent was evaporated in vacuo under a stream of dry hydrogen, and the remaining precipitate was washed thoroughly with dry ether and ethanol, and dried. A white powder was obtained on each polymer, the yield of which was 106% for I″; 117% for II″, 117% for III″, and 138% for IV″, where I″, II″, III″ and IV″ are the corresponding products from the polymers I, II, III and IV, respectively. These were all insoluble in water and in methanol, and I″ and IV″ are mostly, while II″ and III″ are partly soluble in aqueous potassium hydrogen carbonate. After dissolution was attained by standing in aqueous triethylamine for several hours, each solution was concentrated in a vacuum desiccator over conc. sulfuric acid. To

the syrups thus obtained, acetone and a few drops of acetic acid were added and the precipitated polymers were washed with acetone, methanol and ether. After drying, the purified polymers were obtained in 85~95% of the theoretical yields. The final products of I'' and II'' were insoluble in water and soluble in aqueous potassium hydrogen carbonate, and III'' and IV'' were soluble in water. All the solutions gave strong positive biuret reactions. After drying at 108°C/2 mm.Hg over phosphorus pentoxide for five hours, the samples became insoluble, but could readily be turned soluble by heating in aqueous potassium hydrogen carbonate. The observed analytical data were as follows—I'': C, 44.5; H, 5.9; N, 11.3. II'': C, 43.7; H, 6.3; N, 12.1. III'': C, 43.2; H, 6.4; N, 13.2. IV'': C, 44.8; H, 5.7; N, 14.5%.

Copoly-1 : 1 : 2-(γ-benzyl L-glutamate, ε-N-carbobenzoxy-L-lysine, *O*-benzyl-DL-serine) (V).—A mixture of 1.315 g. (5 m mol.) or γ-benzyl N-carboxy-L-glutamate anhydride, 1.530 g. (5 m mol.) of α-*N*-carboxy-ε-*N*-carbobenzoxy-L-lysine anhydride and 2.210 g. (10 m mol.) of *N*-carboxy-*O*-benzyl-DL-serine anhydride in a mixture of 47 ml. of chlorobenzene and 3 ml. of pyridine was sealed in a glass tube. The mixture was heated at 100°C until dissolved, and then allowed to stand for several days at 40°C. The solution gradually increased in viscosity and finally became opalescent gel. The polymer, precipitated on addition of petroleum ether, was thoroughly washed with methanol. After being reprecipitated from chloroform and methanol, the polymer was obtained as a white powder. Yield 3.85 g. (92% of the theor.)

Anal. Found: C, 65.4; H, 6.6; N, 8.3. Calcd. for $[(C_{12}H_{13}NO_3), (C_{14}H_{18}N_2O_3), 2(C_{10}H_{11}NO_2)]_n$: C, 66.1; H, 6.4; N, 8.4%.

The polymer was soluble in chloroform, dioxane, tetrahydrofuran, pyridine and dichloroacetic acid, partially soluble in acetone, and was insoluble in alcohol, ether, petroleum ether and water. It was partially soluble, with swelling, in hot acetic acid.

Copoly-1 : 1 : 2-(L-glutamic acid, L-lysine, DL-serine).—A solution of 3.00 g. of polymer V in 24 ml. of dichloroacetic acid was treated with 5 g. of phosphonium iodide. The reaction product was precipitated by the addition of about 100 ml. of dry ether, and the precipitate was washed repeatedly with dry ether, and then 2.4 g. of a yellowish powder was obtained. This powder was dissolved in methanol and a small amount of insoluble orange-brownish precipitate, included in the product, was almost all removed by centrifugation, and further, the trace of this matter remaing in the solution was precipitated by the addition of a small amount of ether. To the almost colorless clear solution, thus obtained, was added a solution of 0.9 g. of triethylamine in methanol; the resulting precipitate was washed repeatedly with methanol containing a small amount of triethylamine until no iodide ion was detected in the polymer by Beilstein test and by Volhardt method. The free polypeptide was obtained as a white powder. Yield 1.07 g. (68% of the theor.)

Anal. Found: C, 46.4; H, 6.1; N, 15.4; Amino-N, 3.0. Calcd. for $[(C_5H_7NO_3), (C_6H_{12}N_2O), 2(C_3H_5NO_2)]_n$: C, 47.3; H, 6.8; N, 16.2; Amino-N, 3.2%.

This free polypeptide was soluble in water, dilute mineral acids and glacial acetic acid, slightly soluble in methanol and ethanol, and insoluble in acetone and ether. The aqueous solution showed a strong biuret reaction. The viscosities at 25°C and the optical rotations were measured on the solutions having the same concentration of polymers, i.e. 0.464 g./100 ml., and various pH values made by appropriate amounts of hydrochloric acid or sodium hydroxide.

Summary

1. Preparation of four copoly-(L-glutamic acid, DL-serine), the molar ratios of the two amino acid residues were 4:1, 3:2, 2:3 and 1:4, by debenzylation of the corresponding four copoly-(γ-benzyl L-glutamate, *O*-benzyl DL-serine), was attempted.

2. The inter- and/or intra-molecular ester-linkages, formed during the debenzylation reaction between hydroxyl group of serine residue and carboxyl group of glutamic acid residue, was broken by treating with aqueous triethylamine without accompanying racemisation or hydrolysis of the peptide bond.

3. Copoly-(L-glutamic acid, DL-serine) became freely soluble in water when the content of serine residue was more than about 50 mole per cent.

4. Copoly-1 : 1 : 2-(L-glutamic acid, L-lysine, DL-serine), prepared by debenzylation from the parent copolymer, was soluble in water over a whole pH range, and the pH dependence of viscosity and of optical rotation was measured on its solutions. It was found that these properties are very similar to those of natural albumins.

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Spectrochemical Study of Microscopic Crystals. XVIII[1]*. The Color and the Structure of Compounds of a Magnus Green Salt Type*

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(Received May 13, 1958)

Magnus green salt having an empirical formula of $[Pt(NH_3)_4][PtCl_4]$ is peculiar in that the salt in the crystalline state is colored green, whereas the separate component complex-ions in solution are colorless and red, respectively. In order to elucidate the peculiarity, the present authors[2] formerly determined the dichroism of the compound, and concluded that the unusually deep color of Magnus green salt might be due to the possible interaction between platinum atoms which are arranged in a straight line.

When the ammonia molecules in Magnus green salt are replaced by molecules of an alkylamine, the condition around the platinum atoms is supposed to change electronically as well as sterically. Therfore, it seemed to be interesting to see what change would occur in the structure of compounds of a Magnus green salt type with several alkylamines. This paper presents the results obtained concerning the above problem on the basis of dichroism measurements in the visible and ultraviolet region.

Experimental

Materials.—Tetrakis-(methylamine)-platinum-(II) chloride was prepared in colorless crystals from potassium tetrachloroplatinate(II) and an excess of methylamine.

Tetrakis-(ethylamine)-platinum(II), tetrakis-(*n*-propylamine)-platinum(II) and tetrakis-(*n*-butylamine)-platinum(II) chloride were prepared in colorless crystals by the same method as in the case of methylamine. The reaction in every case proceeded in quite a similar way. The crystals were purified by repeated recrystallization from hot water. The solubility of the complex compounds in water seems to decrease as the number of carbon atom increases. All the amines used in the present work were of reagent grade.

Compounds of a Magnus green salt type, $[Pt(amine)_4][PtCl_4]$, were prepared by adding a solution of a tetramine-platinum(II) compound into a solution of potassium tetrachloroplatinate-(II). The crystals of the compounds precipitated at once when the solutions of the two compounds were mixed together.

$[Pt(methylamine)_4][PtCl_4]$ forms green, needle-like crystals. The crystals show a marked dichroism on the prism face; they are dark green with polarized light having its electric vector along the needle-axis, and pale green with the electric vector perpendicular to the axis. The dichroism measurements were made on the prism face with the electric vectors along the two directions.

$[Pt(ethylamine)_4][PtCl_4]$ forms pink, needle-like crystals, which show a straight extinction and are dichroic. They are pink with the electric vector perpendicular to the needle-axis, and very pale pink with the electric vector along the needle-axis. The dichroism measurements were carried out with the electric vectors along the two directions.

$[Pt(n\text{-propylamine})_4][PtCl_4]$ and $[Pt(n\text{-butylamine})_4][PtCl_4]$ form pink, microcrystalline aggregates. The crystals obtained were so small that the dichroism measurements were not made.

Measurements.—The dichroism measurements in the visible and near-ultraviolet region of the crystals were carried out by Tsuchida-Kobayashi's microscopic method[3], as in the former papers of this series. The measurements were also made with a newly constructed apparatus, which consists in principle of a monochromator, a reflecting microscope and a photo-electric detector with a photomultiplier. Most of the parts were made by the Olympus Co.[4] The detailed description of the apparatus will be presented elsewhere.

The *c*- and *a*- absorption in the figures represent the absorption with the electric vector along and perpendicular to the needle-axis of the crystal, respectively.

Results and Discussion

The results of the measurements are shown in Fig. 1 and Fig. 2. It is seen that $[Pt(methylamine)_4][PtCl_4]$ in the crystalline state absorbs the light in a wavelength region much longer than the component complex-ions in solution (Fig. 1).

1) Paper XVII of this series, S. Yamada, H. Nakamura and R. Tsuchida, This Bulletin, **31**, 303 (1958).

2) S. Yamada and R. Tsuchida, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **70**, 142 (1949); S. Yamada, *J. Am. Chem. Soc.*, **73**, 1579 (1951).

3) R. Tsuchida and M. Kobayashi, "The Color and the Structure of Metallic Compounds," Zoshindo, Osaka, 1944, p. 180.

4) R. Tsuchida and S. Yamada, to be published later. The apparatus was constructed in co-operation with the staffs of Olympus Co., to whom we are greatly indebted.

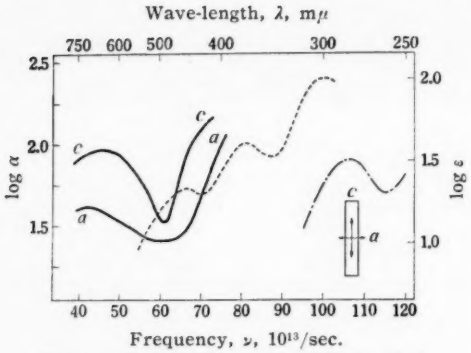

Fig. 1. Absorption spectra of [Pt(methylamine)$_4$][PtCl$_4$] in the crystalline state (——), [Pt(methylamine)$_4$]Cl$_2$ (– – –) and K$_2$[PtCl$_4$] (-----) in aqueous solution. The latter two curves are taken from H. Yoneda, This Bulletin, **30**, 130 (1957) and ref. 6, respectively.

Thus the Magnus green salt type compound of methylamine shows a band at 46.6×10^{13}/sec. (644 mμ), whereas the separate component ions are transparent in this region. It is presumed, therefore, that the complex-ions in the crystal of [Pt(methylamine)$_4$][PtCl$_4$] are under an effect, which is much stronger than that due to the crystalline field of ordinary compounds. The crystal structure analysis of the methylamine Magnus salt is not available for the present. The nature of the effect, however, may be judged from comparison of the dichroism of the compound with the dichroism of Magnus green salt, [Pt(NH$_3$)$_4$][PtCl$_4$][2]. The comparison reveals that the dichroism of the former is quite similar to that of the latter. Therefore, the state of the component complex ions in both the compounds may be almost the same.

It would be appropriate to recall the results with the latter[2], in the crystal of which the planar complex-ions were determined with X-rays to be arranged parallel to each other with platinum atoms disposed at an equal distance along a straight line. The crystal of [Pt(NH$_3$)$_4$][PtCl$_4$] was found to show in the longer wave-length region an absorption band of a new sort, which was not shown by either of the separate component complex-ions alone. For the new absorption band, the absorption is considerably stronger in the direction perpendicular to the planar complex-ions than along the complex-ions. This relationship, combined with the crystal structure analyses[5], led us to the conclusion that in the crystal of Magnus green salt there would be a direct type of interaction between the platinum atoms along the direction perpendicular to the complex-plane.

Based on this result and on the close similarity in the dichroism between the two compounds, the dichroism of [Pt(methylamine)$_4$][PtCl$_4$] in the crystalline state, as has been determined in the present work, may be readily understood, when it is assumed that the *c*-absorption corresponds to the absorption with the electric vector perpendicular to the complex-plane and the *a*-absorption to the absorption with the electric vector along the complex-plane. Thus it may be most likely that the planar complex-ions in the crystal of this compound are arranged in such a way that they are piled one just above and one below another with their planes parallel to each other, and that there would exist a direct type of interaction between platinum atoms in the direction along the needle-axis.

On the contrary, the absorption spectra of [Pt(ethylamine)$_4$][PtCl$_4$] in the crystalline state may be understood in terms of the absorption spectra of the separate component ions undergoing no extraordinary effect. It is seen in Fig. 2 that the

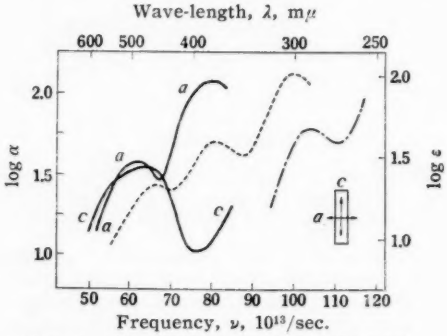

Fig. 2. Absorption spectra of [Pt(ethylamine)$_4$][PtCl$_4$] in the crystalline state (——), [Pt(ethylamine)$_4$]Cl$_2$ (-----) and K$_2$[PtCl$_4$] (----) in aqueous solution. The last two curves are taken from the references cited in Fig. 1.

ethylamine Magnus salt does not show an absorption band in the region of longer wave-length than the region where either of the separate complex-ions alone absorbs

5) E. G. Cox, F. W. Pinkard, W. Wardlaw and G. H. Preston, *J. Chem. Soc.*, **1932**, 2527; M. Atoji, J. W. Richardson and R. E. Rundle, *J. Am. Chem. Soc.*, **79**, 3017 (1957).

in solution. Moreover, the dichroism as well as the absorption spectrum of the crystal closely resembles that of $K_2[PtCl_4]$ in the crystalline state[6]. Thus it may be assumed that the *c*-absorption of the former represents the feature of the $\perp$-absorption and the *a*-absorption the $\parallel$-absorption of the latter, where the $\parallel$- and the $\perp$-absorption denote the absorption with the electric vector parallel and perpendicular to the plane of the complexion. The crystal structure analysis has not yet been reported with this salt. The above consideration indicates, however, that between platinum atoms in the crystal of $[Pt(ethylamine)_4][PtCl_4]$ there is no interaction of such a sort as exists in the crystal of Magnus green salt and the corresponding compound of methylamine.

Summarizing the results obtained in the present and former reports, we may derive the following presumption. *The compound of a Magnus salt type, which is represented by* $[Pt(amine)_4][PtCl_4]$, *may be classified into two kinds. One is colored green, involving a direct type of metal-to-metal interaction. The other is pink and involves no interaction of the above kind.*

The above conclusion may be used for differentiating the two types. In fact, this criterion has been applied in the present work to the case of higher alkylamines. The Magnus salt type compound of *n*-propylamine is obtained in pink microcrystals by the same method as in the case of Magnus green salt. Thus a difficultly soluble precipitate of [Pt(*n*-propylamine)$_4$][PtCl$_4$] is formed at once, when an aqueous solution of [Pt(*n*-propylamine)$_4$]Cl$_2$ is added to a solution of $K_2[PtCl_4]$. The pink color of the salt may be regarded on the basis of the above-mentioned criterion as showing that the compound involves no metal-to-metal interaction. It is concluded in a similar way that no metal-to-metal interaction is operative in the crystal of [Pt(*n*-butylamine)$_4$][PtCl$_4$].

It may well be presumed further that *no metal-to-metal interaction would be present in the crystals of the compounds of a Magnus salt type with higher homologues of alkylamines.* The conclusion which has been obtained up to the present is shown in Table I. It is worth noting that the drastic change from methylamine to ethylamine or higher alkylamines is found.

6) S. Yamada, H. Yoneda and R. Tsuchida, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **69**, 145 (1948).

TABLE I

THE METAL-TO-METAL INTERACTION IN THE COMPOUNDS OF A MAGNUS SALT TYPE, $[Pt(amine)_4][PtCl_4]$

Amine	Color	Pt-to-Pt interaction
NH_3	green	present
CH_3NH_2	green	present
$C_2H_5NH_2$	pink	absent
n-$C_3H_7NH_2$	pink	absent
n-$C_4H_9NH_2$	pink	absent

This result may be explained tentatively in terms of the following two effects. One is due to the change in the inductive effect of the alkyl-group upon the nitrogen atom, and the other to the change in the steric condition around the platinum atom.

The inductive effect, on the one hand, seems to be inadequate for explaining the transition as mentioned above. The tendency of the alkyl-group to push the electronic density toward the nitrogen atom becomes greater as we go from methyl- to higher alkyl-groups. Since higher electronic density on the platinum atom would be favorable for the metal-to-metal interaction, the tendency, as is observed in Table I, can not be explained as due to the inductive effect of the alkyl-group.

On the other hand, the steric condition is considered to become less favorable for the suitable arrangement of the planar complexes, as the alkyl-groups become more bulky. Although increasing number of carbon atoms would not absolutely prevent the platinum atoms from approaching each other so closely that the metal-to-metal interaction might result, it would certainly make difficult the formation of the crystal lattice consisting of series of platinum atoms which are arranged along straight lines.

We have also found that the direct type of interaction between platinum atoms is not present in the crystals of $[Pt\,en_2][PtCl_4]$ or $[Pt(pyridine)_4][PtCl_4]$[7]. This conclusion may be understood in terms of the steric factor, similarly to the case of alkylamines.

Summary

The color and the structure of the compounds of a Magnus green salt type, $[Pt(amine)_4][PtCl_4]$, have been studied. The dichroism with crystals of $[Pt(methylamine)_4][PtCl_4]$ and $[Pt(ethylamine)_4][PtCl_4]$ has been quantitatively determined

7) S. Yamada and R. Tsuchida, to be published later.

at room temperature in the visible and ultraviolet region by the microscopic method. From the measurements, the unusually deep color of the former has been shown to be ascribed to a direct type of interaction between platinum atoms. No interaction of this sort is present in the crystal of the latter, which is pink in color.

It is suggested that the compounds having a formula of $[Pt(alkylamine)_4][PtCl_4]$ may generally be classified into two kinds, one being colored green and the other pink. In the crystals of the former there is a direct type of interaction between platinum atoms at the center of the planar complexes, but no interaction of such a sort is operative in the crystals of the latter.

We wish to thank the Ministry of Education for a grant, which enabled us to purchase the reflecting microscope and accessories. We also thank Dr. Miyata, Dr. Noma and other members of the staff of the Olympus Co. for their co-operation in setting up the apparatus.

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The Ignition of Hydrogen-Oxygen Mixture by Shock Wave. I. The Condition for Shock Ignition for Hydrogen-Oxygen Mixture

By Momotaro SUZUKI, Hajime MIYAMA and Shiro FUJIMOTO

(Received April 10, 1958)

Although the ignition of hydrogen-oxygen mixture by shock wave has been investigated by several authors[1-3], there are not complete data about the shock ignition under various pressures of a gaseous mixture. Therefore, a series of experiments was carried out by the authors of this paper to determine the limit concentration for the gaseous hydrogen-oxygen mixture of various pressures in a shock tube.

The experimental method and procedure are almost the same as in the previous report[4]. Commercial oxygen and hydrogen in cylinders were mixed to a desired composition, and the mixture was kept in a tank over 24 hours, to make the mixing complete.

Results and Discussion

We have carried out a series of experiments on ignition by shock wave against certain gaseous mixtures of definite composition and pressure. The lowest pressure necessary for the ignition is called minimum ignition pressure P_1 for the gaseous mixture of a definite composition

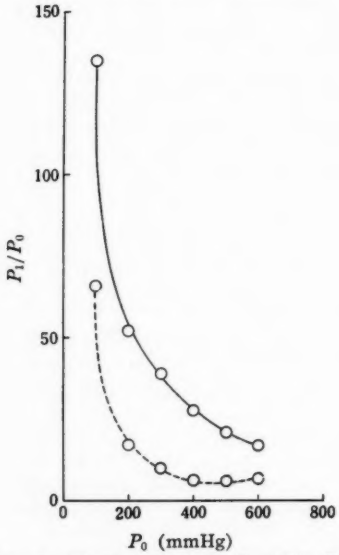

Fig. 1. Variation of P_1/P_0 with P_0, in the case of 80.2% H_2.
——, plane end; ------, conical end.

1) J. A. Fay, "Fourth Symposium on Combustion", The Williams Wilkins Co., Baltimore (1953), p. 501.
2) D. J. Berets, E. F. Greene and G. B. Kistiakowsky, *J. Am. Chem. Soc.*, **72**, 1086 (1950).
3) M. Steinberg and W. E. Kaskan, "Fifth Symposium on Combustion", The Williams and Wilkins Co., Baltimore (1955), p. 664.
4) M. Suzuki, H. Miyama and S. Fujimoto, This Bulletin, **31**, 232 (1958).

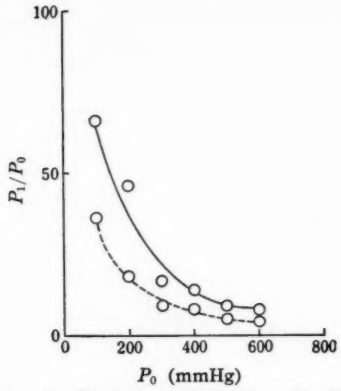

Fig. 2. Variation of P_1/P_0 with P_0, in the case of 66.8% H_2.
——, plane end; ------, conical end.

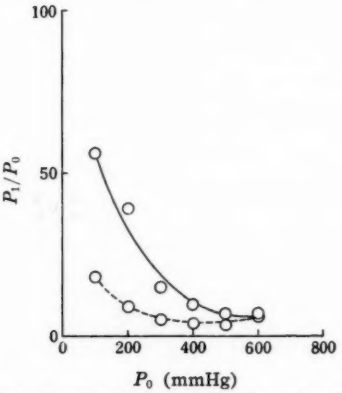

Fig. 3. Variation of P_1/P_0 with P_0, in the case of 48.9% H_2.
——, plane end; ------, conical end.

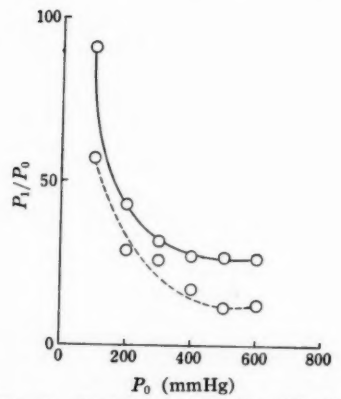

Fig. 4. Variation of P_1/P_0 with P_0, in the case of 10.4% H_2.
——, plane end; ------, conical end.

and a definite pressure P_0. In Figs. 1—4 the ratio P_1/P_0 is plotted against various pressures P_0 of the gaseous mixture of a definite composition. The thick curves correspond to the experiment in which a plane bottom is used in the test chamber and on the other hand the dotted ones for a conical bottom having a vertical angle of 90°, respectively. As shown in these figures, it will be deduced that the higher the pressure P_0, the easier the ignition, and that the ignition is easier in the case of a conical bottom than by the use of a plane bottom.

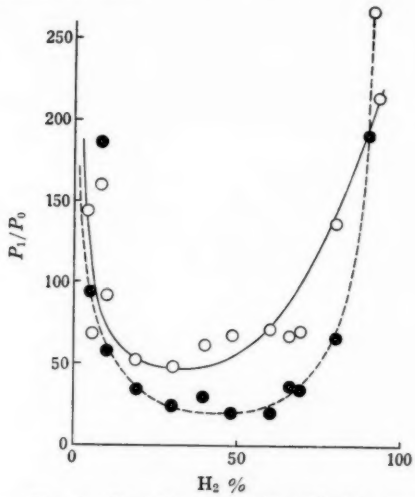

Fig. 5. Variation of P_1/P_0 with the concentration of H_2, when $P_0=100$ mmHg.
——, plane end; ------, conical end.

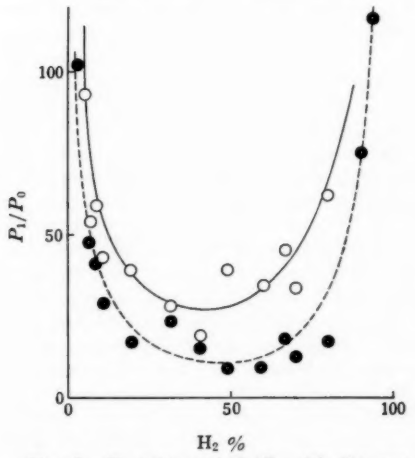

Fig. 6. Variation of P_1/P_0 with the concentration of H_2, when $P_0=200$ mmHg.
——, plane end; ------, conical end.

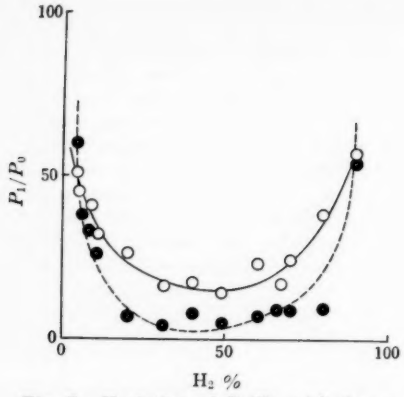

Fig. 7. Variation of P_1/P_0, with the concentration of H_2, when $P_0=300$ mmHg. ——, plane end; ------, conical end.

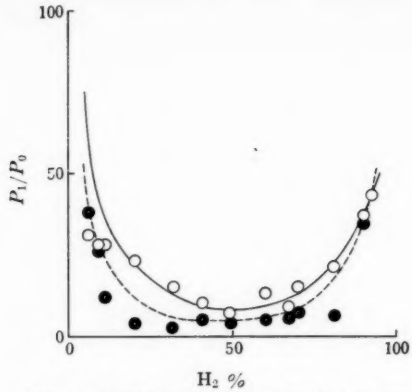

Fig. 9. Variation of P_1/P_0 with the concentration of H_2, when $P_0=500$ mmHg. ——, plane end; ------, conical end.

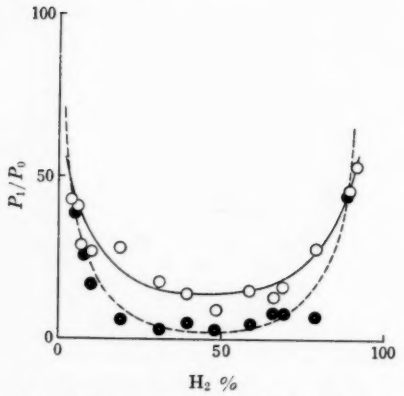

Fig. 8. Variation of P_1/P_0 with the concentration of H_2, when $P_0=400$ mmHg. ——, plane end; ------, conical end.

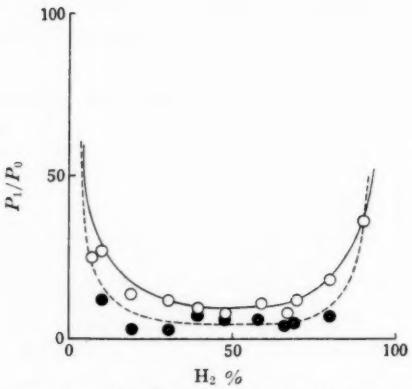

Fig. 10. Variation of P_1/P_0 with the concentration of H_2, when $P_0=600$ mmHg. ——, plane end; ------, conical end.

In Figs. 5—10, the ratio P_1/P_0 is plotted against various compositions of hydrogen and oxygen of definite pressure P_0. For all the diagrams except the case of $P_0=$ 100 mmHg, the minimum ignition point observed corresponds to the gaseous mixture of about 40~50% hydrogen. The limit of explosion of the gaseous mixture, so far as it can be measured, seems to lie in the region from 4 to 94% hydrogen.

Since the reproducibility of the experimental values of the minimum ignition pressure P_1 is not very good, the accuracy of the experimental values is ca. ±10%. These errors seem to cause the irregularity of the measured values of P_1/P_0 in Figs. 5—10.

In our experiments, the ignition temperatures have not been determined directly. Although they may be estimated by means of the same calculations which were described in the previous paper[4], we will postpone the discussion about this problem until the results of further investigation can be obtained using a shock tube equipped with pick-ups, which can be used for measuring the pressure and the velocity of the shock or detonation wave.

Summary

Using the shock tube, the authors measured the minimum ignition pressure P_1 of the reservoir for the hydrogen-oxygen mixture of various pressures (P_0) and various compositions, and obtained the following results.

1) The higher the value of P_0, the easier the ignition.

2) The concentration range of hydrogen in the gaseous mixture for shock ignition is 4~94%, and the minimum value of P_1/P_0 was found in the gaseous mixture of about 40~50% hydrogen, when P_0 is over 200 mmHg.

3) The ignition is easier by the use of a shock tube with a conical end rather than that with a plane end.

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The Ignition of Hydrogen-Oxygen Mixture by Shock Wave. II. Measurement of the Pressure and the Velocity of Shock or Detonation Wave

By Momotaro SUZUKI, Hajime MIYAMA and Shiro FUJIMOTO

(Received April 15, 1958)

In the previous paper[1] of this series, we described the results on the shock ignition of hydrogen-oxygen mixture using a shock tube of rather simple construction. Further investigation with a more complex device will be preferred to get more detailed knowledge. In this report some investigations were made to get fundamental information on the shock ignition of this mixture with a shock tube, equipped with pick-ups of barium titanate for the sake of measurements of pressure as well as of velocity of shock or detonation wave.

Experimental

A brief scheme of the measuring system is given in Fig. 1. As is shown in the figure, the shock tube made from mild steel has a square cross section with an inside lateral of 5 cm. The tube is divided into two portions, the reservoir chamber of A of 60 cm. length and the ignition test chamber C of 180 cm. length respectively. The chambers A and C are separated by several sheets of cellophane films, which can be punctured by the needle N through a side tube attached obliquely to the central axis of the shock tube.

For the measurement of pressures, five checking stations which are denoted by P_1, P_2, P_3, P_4 and P_5, are located on the wall of the test chamber. These stations are placed at a distance of 20 cm. from each other. The measuring equipment of each station is a pick-up (Fig. 2) consisting of ceramics of barium titanate whose natural frequency is something over 10 kc. A pick-up at P_0, placed near the membrane, is used for starting the sweep of the oscillograph.

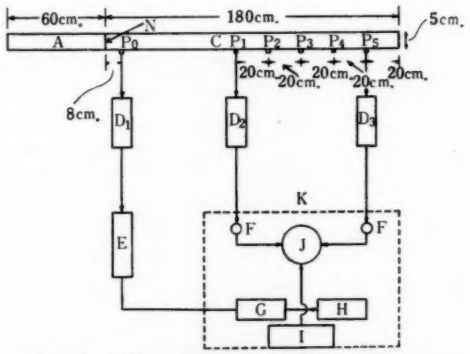

Fig. 1. Scheme of the measuring system.
A, Reservoir chamber;
C, Test chamber;
D_1, D_2 and D_3, Pre-amplifiers;
E, Trigger amplifier;
F, Signal input;
G, Sweep trigger;
H, Sweep oscillator:
I, Time-mark-oscillator;
J, Cathode-ray tube;
K, Two-element-oscillograph;
N, Needle;
P_0~P_5; Pick-up stations.

Just as the shock wave starts at the puncturing of the diaphragm, the pick-up at P_0 operates, and accordingly the signal from this pick-up will be transmitted through the pre-amplifier D_1 to the trigger amplifier E which operates the sweep-trigger G. Then the sweep-oscillator H of the oscillograph K will start and will give a horizontal time axis on the oscillogram. As the wave further propagates and passes the pick-up stations P_1 etc., the pressure change which is caused by the wave passing there will register on the pick-ups and this transient pressure change will

1) M. Suzuki, H. Miyama and S. Fujimoto, This Bulletin, **31**, 816 (1958).

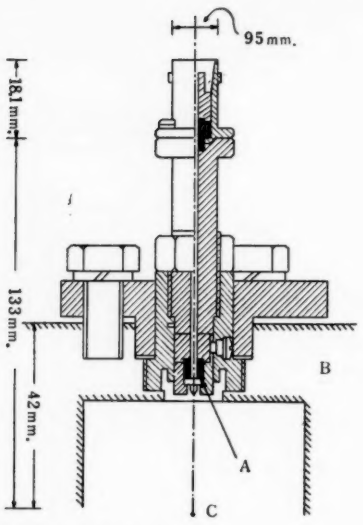

Fig. 2. Pick-up.
A, Barium titanate;
B, Wall of the shock tube;
C, Center of a cross section of the shock tube.

show itself as a displacement along the vertical axis of the oscillogram whose height gives the value of the pressure change. As we have a synchronous time marking by means of oscillator I in each oscillogram, we can evaluate the time delay of these successful pressure signals and then estimate the average velocity of the wave between two subsequent stations accordingly.

The characteristics of the apparatus used are given below. The pre-amplifiers have input-impedance 5 MΩ, output-impedance 100 kΩ, gain 53 db, maxium deviation of gain −2 db between 2c./s. and 20 kc./sec., and noise ratio less than −60 db. The trigger amplifier has output-impedance 100 kΩ, gain 40 db, maximum deviation of gain −2 db between 1 kc./sec. and 20 kc./sec., trigger-output 75 V_{p-p}, trigger-delay 0.6 msec.∼1.3 msec. which on requirement can be reduced to less than 2 μsec. by cutting off the circuit of the trigger-delay. The scale of the time-mark oscillator can be varied in four steps i.e. 10 msec., 1 msec., 100 μsec. and 10 μsec., and the error of scale is less than 5%. The two-element oscillograph has the following characteristics. Cathode-ray tube is the type 130 SB11 (Toshiba). The amplifier has an input-impedance ca. 2 MΩ, gain ca. 70 db, maximum deviation of gain ±3 db between DC and 500 kc./sec., input-level about 1 mV∼100 V, and continuous variable attenuator 1:1 to 1000:1. The sweep-trigger and sweep-oscillator have input-level ca. 10 mV_{p-p}, and time of duration for a single-sweep 100 msec./cm.∼10 μsec./cm.

The pick-up used for measurement was calibrated by means of the device shown in Fig. 3. The procedure of calibration is as follows. The

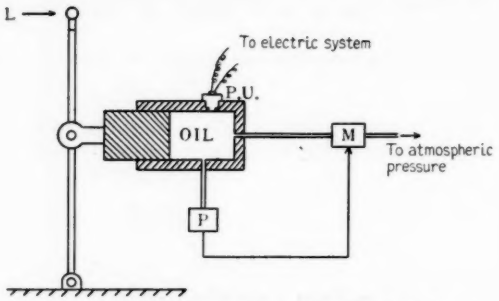

Fig. 3. Apparatus for calibrating pick-up.
P, Pressure switch;
M, Electro-magnetic valve;
L, Lever

pressure of the vessel, which is filled with low viscosity oil, will increase by pushing the lever L. When the pressure attains a certain definite value on which the pressure switch P is adjusted, electrically operate the valve M, which is suddenly opened. Accordingly, the pressure inside the vessel is reduced to atmospheric pressure during a very short period, and this pressure change is recorded through the pick-up in the oscillogram. We can calibrate the displacement of the oscillogram at a certain value of pressure by comparing this with the scale of the pressure switch which is previously standardized by an absolute manometer.

Other details about the experiment which are not described in this paper will be found in our previous papers[1,2] or elsewhere.[3]

Results and Discussion

A picture of an oscillogram of a propagating shock wave in the test chamber, which is filled only with air, is shown in Fig. 4. The picture was taken for the sake of visualizing the real behavior of a shock wave propagating in a tube filled with air without initiating any chemical reaction. The variation of the pressure P and the velocity of the shock wave with P_1/P_0 is obtained from many such pictures as this and shown in Figs. 5 and 6, where P_1 and P_0 are the pressures of the reservoir and the test chambers respectively. The values of P_0 are always atmospheric pressure. The experimental values of P are rather greater than the theoretical

2) M. Suzuki, H. Miyama and S. Fujimoto, This Bulletin, **31**, 232 (1958).

3) R. B. Morrison, "A Shock-Tube Investigation of Detonative Combustion", Engineering Research Institute, Univ. of Michigan, Ann Arbor (1955); P. F. W. Geiger and C. W. Mautz. "The Shock Tube as an Instrument for the Investigation of Transonic and Supersonic Flow Patterns", Engineering Research Institute, Univ. of Michigan, Ann Arbor (1949): P. M. Steinberg and W. E. Kaskan, "Fifth Symposium on Combustion", The Williams and Wilkins Co., Baltimore (1953), p. 664.

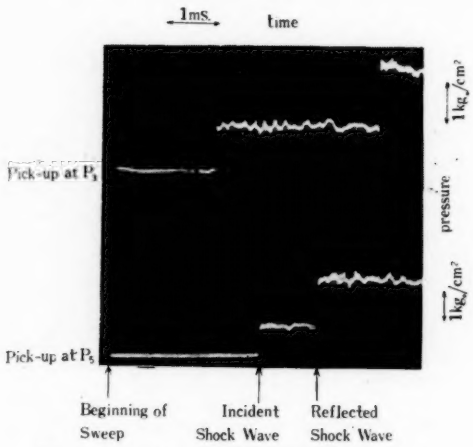

Fig. 4. A picture of oscillogram, when the test chamber is filled with air. P_0=760 mmHg, P_1/P_0=5.

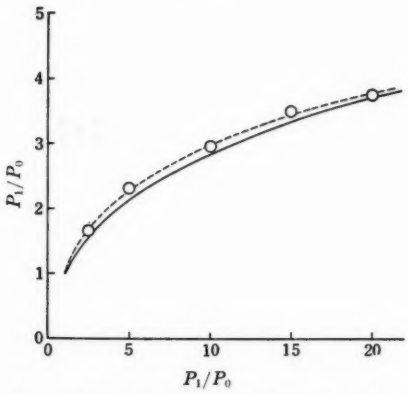

Fig. 5. Variation of the pressure of the incident shock wave with P_1/P_0
------ experimental value;
—— theoretical value.

values as shown in Fig. 5. The discrepancies probably lie within the limit of characteristic error of the pick-up used. As may be seen in Fig. 6, the experimental values of the velocity of shock wave are smaller than those expected from the theoretical considerations, and this tendency looks more reasonable when compared with the results of any other author.[4]

In the next experiment we have carried on a series of measurements using the

4) R. E. Duff, "The Use of Real Gases in a Shock Tube" Engineering Research Institute, Univ. of Michigan, Ann Arbor (1951).

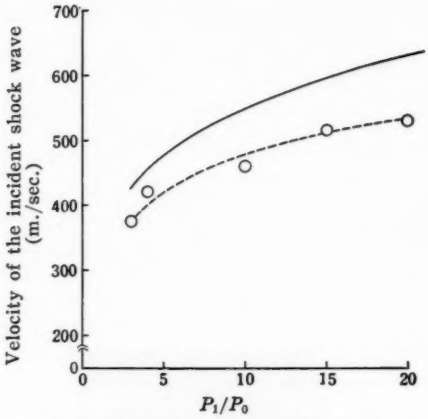

Fig. 6. Variation of the velocity of the incident shock wave with P_1/P_0.
------ experimental value;
——theoretical value.

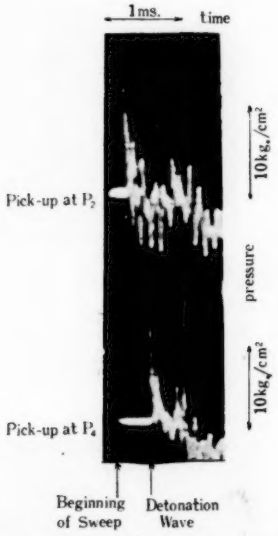

Fig. 7. A picture of oscillogram, when the test chamber is filled with $2H_2+O_2$. P_0=200 mmHg, P_1/P_0=10.1.

gaseous mixture $2H_2+O_2$ in the test chamber. Compared with the former case[1], the ignition was easier, i.e., the ignition pressure P_1 is lower, but the critical value of this pressure was more ambiguous. These results probably occur because the cross-section of this shock tube is larger, the number of sheets of cellophane films under the same pressure-difference, is more, and therefore the effect of puncturing the membrane upon the detonable

gaseous mixture is greater than in the previous shock tube.[1].

A typical oscillogram of pick-up pressures in a series of detonation experiments where the pressure of the detonable gas mixture in the test chamber is more than 200 mmHg, is shown in Fig. 7. In this figure, the pressure of the shock wave is not observable, but only the pressure of the detonation wave can be observed. The same features are observed in oscillograms of all pick-up stations and therefore it is concluded that the detonation was initiated between the membrane and the first pick-up P_1 and that the detonation of the gaseous mixture became stable before the detonation wave arrived at the pick-up P_1.

Under a pressure of 100 mmHg, the detonation took place somewhere along the test chamber. This detonation was initiated by an incident shock wave in some cases and by a reflected one in other cases. The distinction as to whether the cause of initiation is due to the incident or the reflected shock wave, can be told from the relative position of the detonation sign and the shock sign in the oscillograms of the pick-up stations (Figs. 8 and 9).

These differences of the oscillograms between points at high pressure and at

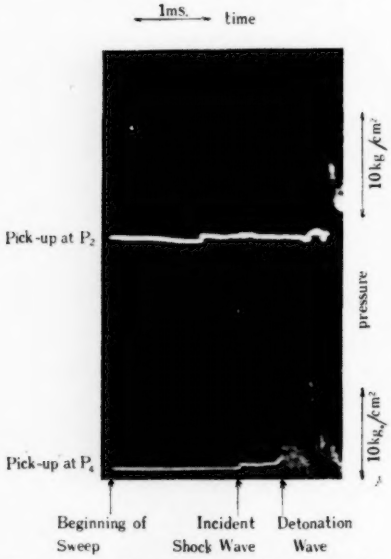

Fig. 8. A picture of oscillogram, when the test chamber is filled with $2H_2+O_2$. $P_0=100$ mmHg, $P_1/P_0=57.0$.

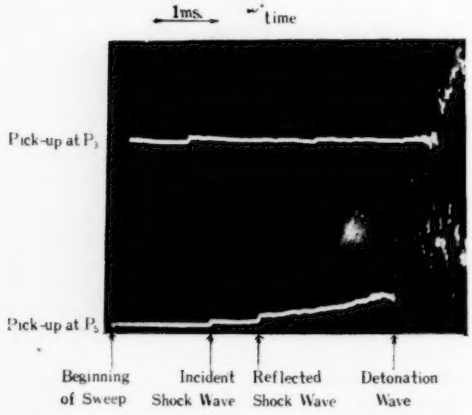

Fig. 9. A picture of oscillogram, when the test chamber is filled with $2H_2+O_2$. $P_0=100$ mmHg, $P_1/P_0=55.8$

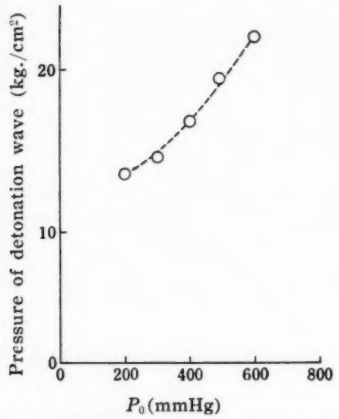

Fig. 10. Variation of the pressure of detonation wave with P_0.

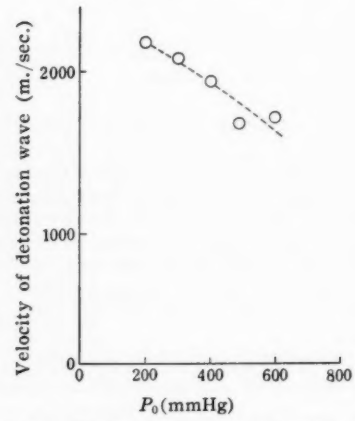

Fig. 11. Variation of the velocity of detonation wave with P_0.

low pressure is perhaps because of the difference of the disturbing effect of fractured membrane fragments in both cases. Although this effect seems to be very little in the latter case, it must be verified by a further investigation.

The variation of the pressure and the velocity of detonation wave with the pressure of the detonable gas mixture, P_0, calculated from the oscillograms, are shown in Figs. 10 and 11. These figures correspond to the cases where P_0 is higher than 200 mmHg. It will be concluded from the results of these experiments that the pressure of the detonation waves increases along with the increase of the pressure P_0, but on the other hand the velocity of waves decreases as the pressure P_0 increases.

Summary

We have carried on a series of experiments on the shock ignition of a gaseous mixture $2H_2+O_2$ by using a shock tube equipped with pick-ups, which can be used for measuring the pressure and the velocity of shock or detonation waves. The results are summarized as follows:

1) When the pressure P_0 of a gaseous mixture is higher than 200 mmHg, the ignition starts between the membrane and the first pick-up along the test chamber and the detonation becomes stable before the detonation wave arrives at the first pick-up.

2) In case P_0 is 100 mmHg, the detonation is initiated by an incident shock wave in some cases and by a reflected one in other cases.

3) When P_0 is higher than 200 mmHg, the pressure of the detonation wave initiated by shock ignition increases along with the increase of P_0 but its velocity decreases with a similar increase.

The authors wish to express their thanks to Dr. Martin Steinberg of the Armour Research Foundation of Illinois Institute of Technology for his helpful advice in constructing our apparatus, and to Mr. Y. Iwai and his staff of Tokyo Shibaura Electric Co. for the construction of our shock tube.

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The Formation of Stable Semiquinone of Methylene Blue by the Irradiation of Visible Light and γ-Rays.

By Masao KOIZUMI and Hiroshi OBATA

(Received May 15, 1958)

According to a recent paper of A. J. Swallow[1], stable semiquinone radical is formed when a deaerated solution of methylene blue in 22.9 N sulfuric acid containing a small quantity of ethanol is irradiated by γ-rays. He considered that methylene blue, in this case, is reduced while ethanol is oxidized.

In connection with Swallow's results, we have taken up the following three questions.

1) Is it not possible to form semiquinone by the irradiation of Swallow's system with the visible light?

According to Swallow's view, ethanol is to be oxidized into aldehyde by OH radical produced from the radiolysis of water, hence semiquinone should remain unaffected by the existing aldehyde. Now it is well known that when alcoholic solution of methylene blue is irradiated by the visible light in vacuo, the dehydrogenation of alcohol by dye-ion takes place, the dye being transformed into the leuco compound. Therefore it is very natural to expect that the visible-light irradiation of Swallow's system will produce a stable semiquinone.

2) Is benzoic acid which is dissolved in concentrated sulfuric acid as a protonated form, more effective than alcohol for preparing semiquinone radical?

1) A. J. Swallow, *J. Chem. Soc.*, **1957**, 1553.

If ethanol acts as OH scavenger as Swallow seemingly supposed, benzoic acid, which is a strong OH scavenger in neutral or alkaline solution, is expected to be so in the case of strong acidic solution. In addition it would be interesting to examine the concentration effect of ethanol from the above viewpoint.

3) Which reaction will predominate when the solution of methylene blue in 22.9 N sulfuric acid but containing no ethanol is irradiated by γ-rays, the formation of semiquinone or the decomposition of methylene blue by OH radical? Or alternatively, does the reaction proceed in quite a different manner?

In case of a neutral solution of methylene blue, decomposition of dye and the formation of leuco compound take place simultaneously, the former being suppressed by the addition of OH scavenger. It therefore seems worth while to examine the case of strong acidic solution.

In order to examine these problems the following experiments were undertaken using a similar system to that of Swallow.

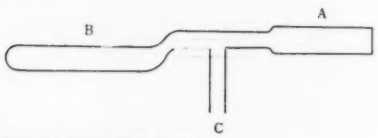

Fig. 1. Reaction cell.
Part A. Thickness 10 mm. plane parallel.
Part B. Diameter ca. 12 mm. (painted with black enamel).

Experimental

The reaction cells used are shown in Fig. 1. The solution is irradiated in Part B, the outside wall of which is painted with black enamel to interrupt the visible light. The absorption spectra of the solution is observed in Part A with the use of Hitachi Spectrophotometer. The solution, when necessary, is evacuated, transferred into B through C, and then the cell is fused off at C.

The irradiation of γ-rays was performed at the Department of Radiology in Tohoku University. Twenty Curie ^{60}Co injecting source was placed 25 cm. away from the samples and all the samples were irradiated for about 15 hours. The dose of γ-rays entering the solution during this period is estimated to be about 21000 Rentgen.

The irradiation of visible light was done by a projection lamp (75 V, 500 W) which was used at 55 V, and placed 4.5 cm. away from the cell.

The procedure for sampling was as follows. The solution of methylene blue 1×10^{-4}M in sulfuric acid (22.9 N) containing 0.001~0.6 M ethanol, for example, was prepared by mixing 0.25 cc. of 2×10^{-3}M aqueous methylene blue solution, 3.18 cc. of pure sulfuric acid and 1.57cc. of an aqueous alcoholic solution of appropriate concentration. The volume contraction during the mixing was disregarded. No special attention was paid to the formation of ester[2].

Below will be given the experimental results and discussions about the afore-mentioned three questions.

Results and Discussion

1) Photochemical Formation of Semiquinone. — The results obtained for the

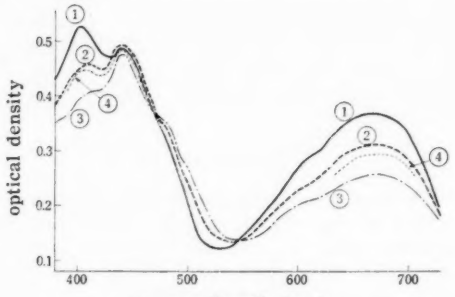

Fig. 2. Change of absorption spectra (visible light irradiation).
Methylene blue ca. 4×10^{-5}M
H_2SO_4 22.9 N
Alcohol 3.38 M
①—— initial
②- - - after 3.5 hr.' irradiation
③—·— after 8 hr.' irradiation
④····· exposed to oxygen after irradiation

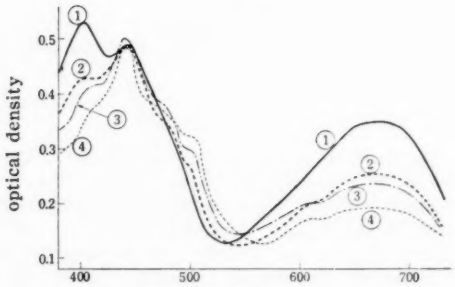

Fig. 3. Change of absorption spectra (visible light irradiation).
Methylene blue ca. 4×10^{-5}M
H_2SO_4 22.9 N
Alcohol 4.96 M
①—— initial
②- - - after 1 hr. irradiation
③—·— after 2.5 hr.' irradiation
④····· after 5.5 hr.' irradiation

2) D. J. Clark and G. Williams, ibid., **1957**, 4218.

following two deaerated solutions are shown in Fig. 2 and Fig. 3.

a) Methylene blue ca. 4×10^{-5}M; sulfuric acid, 22.9 N; ethanol 4.96 M.

b) Methylene blue ca. 4×10^{-5}M; sulfuric acid, 22.9 N; ethanol 3.38 M.

The absorption curves of the illuminated solutions are quite similar to those reported by Swallow and in view of the investigation of Michaelis et al.[3], it can safely be concluded that the stable radical is very easily produced by the photochemical reaction. Perhaps the photochemical method would be a better one for preparing a stable free radical than that employing high energy radiation, since the reaction in the former case would be far more simple than in the latter.

2) Formation of Semiquinone by γ-ray Irradiation.—In Fig. 4 to Fig. 6 are shown the typical results obtained when the deaerated solutions of methylene blue in 22.9 N sulfuric acid containing ethanol of various concentrations or benzoic acid instead of ethanol are irradiated by γ-rays.

As to the effect of the concentration of ethanol, there exists scarcely any difference from 0.1 M up to 0.5 M. Below 0.01 M, the quantity of semiquinone formation seems somewhat smaller, but the reproducibility being not so good, an exact comparison is difficult. Roughly speaking, the effect of concentration is rather small from 0.001 to 0.5 M. The use of benzoic acid in place of ethanol is very effective

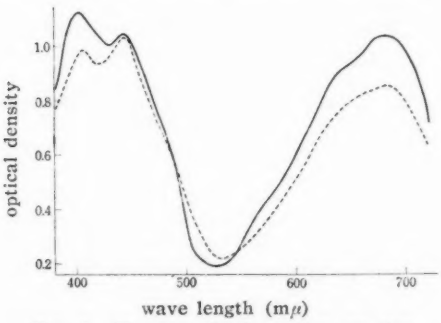

Fig. 4. Change of absorption spectra (γ-rays irradiation, 15 hr.)
Methylene blue 1×10^{-4}M
H_2SO_4 22.9 N
Alcohol 0.01 M
—— initial
····· after irradiation

3) L. Michaelis, M. P. Schubert and S. Granick, *J. Am. Chem. Soc.*, **62**, 204 (1948).

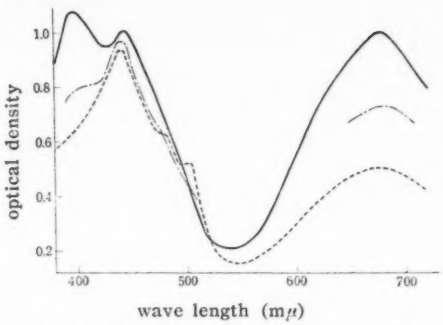

Fig. 5. Change of absorption spectra (γ-rays irradiation, 15 hr.)
Methylene blue 1×10^{-4}M
H_2SO_4 22.9 N
Alcohol 0.5 M
—— initial
····· after irradiation
– – – exposed to oxygen after irradiation

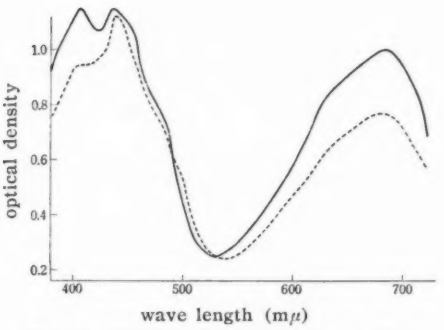

Fig. 6. Change of absorption spectra (γ-rays irradiation 15 hr.)
Methylene blue 1×10^{-4}M
Benzoic Acid 0.005 M
H_2SO_4 22.9 N
—— initial
····· after irradiation

for producing semiquinone and 0.005 M of the acid gives quite a similar result as that of 0.15 M or more of ethanol. Thus it is certain that benzoic acid which in this case must be dissolved as $C_6H_5COOH_2^+$ acts as a good OH-scavenger.

The above results support the view that ethanol acts as a pure OH-scavenger and the necessary condition for the formation of semiquinone is perhaps the existence of OH-scavenger in a certain amount.

3) The Results when no OH-scavenger is added. — When the deaerated solution of methylene blue in 22.9 N sulfuric acid is irradiated by γ-rays, practically no

change is perceived in the original absorption curve. But the solutions of methylene blue in 22.9 N sulfuric acid not deaerated, when irradiated by γ-rays, show a small but definite decrease of absorption throughout the whole visible region as shown in Fig. 7 and Fig. 8, whether it contains 0.1 M ethanol or not. This decrease must undoubtedly be attributed to the destruction of some dye molecules.

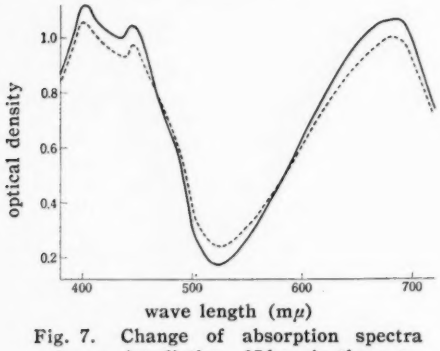

Fig. 7. Change of absorption spectra (γ-rays irradiation, 15 hr., in the presence of oxygen).
Methylene blue 1×10^{-4}M
H_2SO_4 22.9 N
Alcohol 0.1 M
—— initial
····· after irradiation

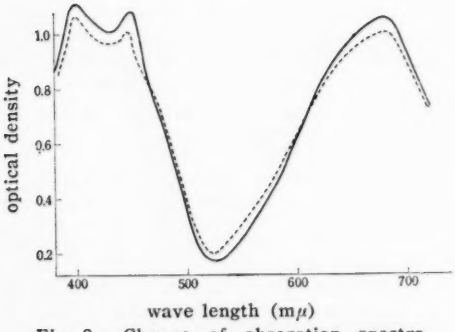

Fig. 8. Change of absorption spectra (γ-rays irradiation, 15 hr., in the presence of oxygen).
Methylene blue 1×10^{-5}M
H_2SO_4 22.9 N
Alcohol not added
—— initial
····· after irradiation

These results are in sharp contrast with those for neutral solutions of methylene blue where the decomposition of dye and the formation of leuco-compound go hand in hand in vacuo. These noteworthy results would be interpreted as follows.

Let us take up the case of the deaerated system at first. If the attack of hydrogen atom and that of OH radical upon dye molecules take place simultaneously, both the formation of semiquinone and the irreversible destruction of dye molecules should occur as is the case in neutral solution. This contradicts the experimental results and the interpretation must be as follows. Hydrogen atom produced by the radiolysis of water first attacks the methylene blue molecule, converting it into semiquinone radical, and following this step, the attack of OH on the latter takes place recovering the original dye molecule. It seems worth while to note that OH attacks the semiquinone radical preferentially and in such a way as to reproduce the original dye, since this behavior is quite different from that of the neutral solution where dye molecules are believed to be attacked at various points by OH radical. In the presence of oxygen, the semiquinone radical intermediately produced would be reoxidized by OH radical (in case of no ethanol) or O_2 molecule (when ethanol exists), and the irreversible destruction would perhaps occur in a common way in the existent oxygen.

The above interpretation that hydrogen first attacks the dye molecule and then the produced semiquinone reacts with OH, seems at first rather curious. But the following consideration will make it very plausible.

The production of H and OH from water is generally believed to occur by the following processes.

$$H_2O \rightsquigarrow H_2O^+ + e \qquad (1)$$

$$\left.\begin{array}{ll} e + H_2O \rightarrow H + OH^- & (a) \\ e + H^+aq. \rightarrow H + aq. & (b) \end{array}\right\} \quad (2)$$

$$H_2O^+ + aq. \rightarrow OH + H^+aq. \qquad (3)$$

In the case of strong acidic solution, the hydrogen atom should be easily produced via 1 and 2b, but the existence of $H^+aq.$ in large quantity would inhibit the reaction 3. Or it may be said that when the reaction 3 takes place, the reverse reaction would at once recover H_2O^+ again. Thus the oxidative radical that exists is not OH but H_2O^+ and this is expected to be removed by ethanol, but the dye ion which exists as a polyvalent cation would not be attacked by H_2O^+ owing to a strong electrostatic repulsion. This causes the reduction by hydrogen atom to proceed and H_2O^+ only attacks the semiquinone

radical and by depriving its labile hydrogen atom stabilizes as H_3O^+. Such would perhaps be a correct interpretation for the fact that the dye is scarcely affected at all when OH scavenger does not exist.

Thus our results can be comprehended very well along Stein's general view[4] for the radiolysis of the aqueous solution.

Summray

In connection with Swallow's observation that methylene blue in 22.9 N sulfuric acid aqueous solution containing a small amount of alcohol is reduced to a stable semiquinone when irradiated with γ-rays in vacuo, some experiments were done to investigate the role of alcohol.

It was elucidated that alcohol acts most certainly as an OH scavenger. Benzoic acid was found to be an effective OH scavenger in this case just as in usual solutions.

In the absence of alcohol either the formation of semiquinone or the irreversible decomposition of dye scarcely ever takes place in vacuo while in the aerated solution, only the latter reaction slowly proceeds. A plausible interpretation of these results was given.

It was further found that the formation of stable semiquinone can easily occur on irradiation of Swallow's system with the visible light.

The authors express their thanks to Dr. Y. Koga, Professor of the Department of Radiology, Tohoku University, for permitting them to use the ^{60}Co injection source.

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4) G. Stein, *Discussions Faraday Soc.*, **12**, 227 (1952).

Polarographic and Coulometric Studies of Tropolone

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(Received April 21, 1958)

Polarographic behavior of tropolone and its derivatives has been studied by several authors[1-5]. James and Speakman[2] studied the polarographic behavior of tropolone, but they used a supporting electrolyte which contained borate ions. It has been confirmed that borate ions form complexes with tropolone. Neish and Müller[3] studied the polarographic behavior of β-methyltropolone and the related compounds and discussed the reduction mechanism, but they did not include tropolone itself in their study. Moreover, no coulometric analysis has been made as far as tropolone and the related compounds are concerned. The mechanisms of the electrode processes of tropolone so far proposed were analogized from those of benzaldehyde.

It seems worth-while, this being the case, to reinvestigate the polarographic behavior of tropolone in buffer solutions containing no borate ion and to determine the number of electron involved in the reduction from the coulometric analysis.

Experimental

Materials.—Tropolone was purified by recrystallization from mixed solvent of petroleum ether and benzene and by successive sublimation. A white needle-like crystalline compound of a melting point of 51°C (uncorrected) was obtained. The buffer solutions used in this study are listed in Table I. All electrolytic solutions contained

1) F. Šantavý, *Collection Czechoslov. Chem. Communs.*, **14**, 145 (1949).
2) J. C. James and J. C. Speakman, *Trans. Faraday Soc.*, **48**, 474 (1952).
3) W. J. P. Neish and O. H. Müller, *Rec. trav. chim.*, **72**, 301 (1953).
4) T. Murayama, Presented at the 7th Annual Meetings of Chem. Soc. Japan in Tokyo (1954).
5) F. Šantavý, B. Jámbor, A. Německova, J. Mollin and J. Bartek, *Collection Czechoslov. Chem. Communs.*, **22**, 1655 (1957).

TABLE I
BUFFER SOLUTIONS USED

pH	Buffer solutions
2.2~ 8.0	Citric acid—disodium phosphate
8.9~11.4	Sodium carbonate —sodium bicarbonate
11.0~12.0	Sodium hydroxide —disodium phosphate
13.0	0.1 M Potassium hydroxide

0.1 M of potassium nitrate as an indifferent electrolyte and also contained 0.01% of gelatin as a maximum suppressor. Commercial guaranteed reagents were used for the buffer and supporting electrolyte without further purification.

pH measurements.—The pH of the solution was measured before and after the measurements with a Hitachi pH meter Model EHP-1 with a glass electrode.

Polarography.—Current-voltage curves were recorded with a Shimadzu photo-recording polarograph Model SH-UI and with a Yanagimoto pen-recording Galvarecorder Model 2 with an automatically scanning applying potentiometer attached.

The dropping mercury electrode used had an m value of 0.893 mg./sec. and a drop time t of 3.94 sec., being measured in 0.1 M potassium chloride solution at 55 cm. of mercury height at 25°C with open circuit. An H-type cell with a saturated calomel electrode was used. Pure nitrogen gas was bubbled through the solution for about 30 minutes to remove oxygen dissolved in the solution. All measurements were carried out in a thermostat of 25.0±0.1°C.

Coulometry.—For the coulometric measurements[6], the solutions whose constituents were the same as those subjected to the polarographic measurement were employed. The potentials of the working electrode were controlled against a saturated calomel electrode during the electrolysis with a Yanagimoto automatic potentiostat. The electrolytic current was recorded with a Yanagimoto pen-recording Galvarecorder Model 2 connected to the circuit in series. The potentials of the working electrode were adjusted to be −1.15 volts vs. S.C.E. at pH 3.0, −1.35 volts at pH 5.35 and −1.55 volts at pH 8.0.

The electrolysis cell was of a beaker type, the diameter being 4.8 cm. and the height, 5.5 cm., which is shown in Fig. 1b. A mercury pool was used for the working electrode and a spiral platinum wire whose effective area was approximately 1 cm^2 was used for the auxiliary anode. The latter was connected to the electrolytic solution through a Hickling-type salt bridge of potassium nitrate. The dropping mercury electrode was also placed in the electrolysis cell, which made it possible to measure the current-voltage curve at any moment during the controlled potential electrolysis. The electrical circuit of the equipment was designed for the purpose, as shown in Fig. 1a. The electrolytic solution was stirred by bubbling pure nitrogen gas and by means of a magnetic stirrer.

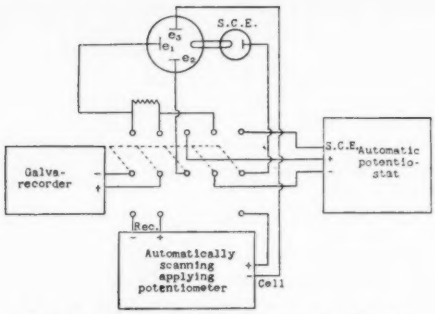


Fig. 1a. Schematical diagram of the circuit for the electrolysis: e_1, working electrode (Hg pool); e_2, auxiliary anode (Pt wire); e_3, dropping mercury electrode.

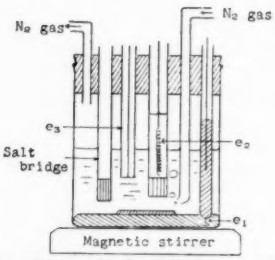


Fig. 1b. Electrolysis cell: e_1, e_2 and e_3, the same as in Fig. 1a.

The concentration of tropolone was varied in the range from 2×10^{-4} M to 1×10^{-3} M. The temperature of the solution was kept at 20°C. The maximum current in the coulometry was 5 milliamperes.

Results

Current-voltage curves.—The current-voltage curves obtained with the solution containing 1×10^{-3} M of tropolone at various pH's are shown in Fig. 2. One wave was observed in the media of pH lower than 4.0, two waves in the pH range from 5 to 7 and one wave in the pH range from 8 to 10. Only an indistinct wave was observed in the pH region higher than 11.

The half-wave potentials of all waves except the second wave at the intermediate pH's were observed to shift to negative potential with the increase of pH. The wave height except that of the second wave decreased with the increase of pH. The half-wave potential and the wave height of the second wave were observed

6) J. J. Lingane, "Electroanalytical Chemistry", Interscience Publishers, Inc., New York (1953), p. 347.

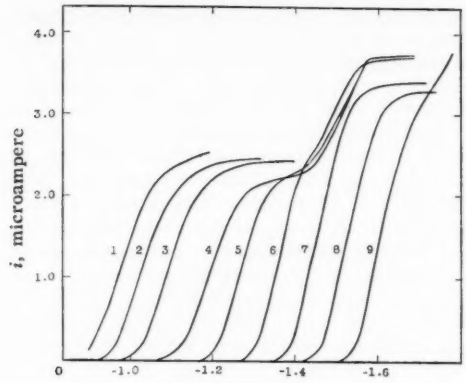


Fig. 2. Current-voltage curves of 1×10^{-3} M tropolone obtained with dropping mercury electrode: 1, pH 2.2; 2, pH 3.0; 3, pH 4.0; 4, pH 5.0; 5, pH 6.0; 6, pH 7.0; 7, pH 8.0; 8, pH 9.0; 9, pH 10.0.

to be almost independent of pH. The variation of half-wave potential $E_{1/2}$ of the first wave with pH was given by

$$E_{1/2} = -0.837 - 0.07\ \mathrm{pH}$$

volt vs. S.C.E. at 25°C.

As seen in Fig. 3, a linear relation was obtained between the concentration and the wave height of the first wave in the pH region of 3.0 to 6.0.

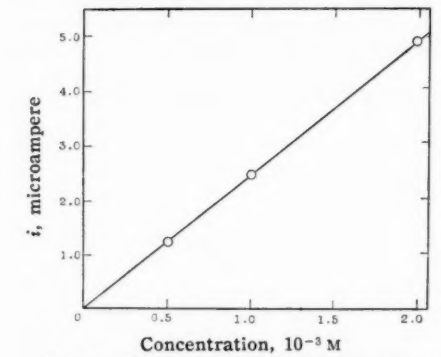


Fig. 3. Relation between the concentration of tropolone and the limiting current obtained at pH 4.0 at 25°C.

Provided that the limiting current is controlled by diffusion, it must be proportional to the square root of the effective height of mercury. The relation was examined with the first and the second waves at three different mercury heights, i. e., 55 cm., 45 cm. and 40 cm. in the pH range of 3.0 to 7.0. The results obtained are listed in Table II, which indicate that the ratio, $i_l/h_{eff.}^{1/2}$, is nearly constant and that the limiting current is of diffusion control.

TABLE II
RELATION BETWEEN THE LIMITING CURRENT AND THE MERCURY HEIGHT

pH	Mercury height*	$i_l/h_{eff.}^{1/2}$ (μ amp. cm$^{-1/2}$)	
		1st wave	2nd wave
3.0	I	0.346	
	II	0.344	
	III	0.347	
4.0	I	0.337	
	II	0.330	
	III	0.332	
5.0	I	0.302	0.213
	II	0.320	0.227
	III	0.341	0.206
6.0	I	0.330	0.209
	II	0.335	0.214
	III	0.341	0.202
7.0	I	0.519	
	II	0.478	
	III	0.456	

* I, h=55 cm.; II, h=45 cm.; III, h=40 cm.
The concentration of tropolone was 1×10^{-3} M.

Coulometric measurements. — Before and after the electrolysis, the polarograms were recorded with the sample solution, so that the initial and the final concentration of tropolone could be estimated from the wave heights. However, the polarogram which was recorded when a half quantity of tropolone was reduced showed two new waves appearing at the potentials more positive than that of the reduction wave of tropolone. A typical example of the polarograms is given in Fig. 4. The first wave is anodic and the second is cathodic, the latter appearing at somewhat more positive potentials than the reduction wave of tropolone. The third wave is consipered to be the wave of tropolone because the potentials are identical with those of tropolone.

The height of the first wave was observed to decrease with the lapse of time; it decreased to nearly a half of the original height after some twenty minutes. The height of the second wave, on the other hand, increased with the lapse of time. This was observed with all solutions whose pH's were between 3 and 8. The half-wave potentials of those new waves were found to depend on pH of the

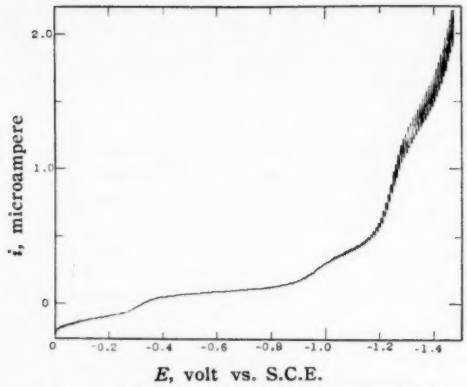


Fig. 4. Current-voltage curve recorded at pH 5.35, at 20°C, with the solution in which a half of tropolone was reduced by controlled potential electrolysis. The initial concentration of tropolone was 3.53×10^{-4} M.

solution and both waves shifted to more negative potentials by 40 to 80 mV. with the increase of one unit of pH.

The quantity of electricity required in the controlled potential electrolysis was determined by integrating graphically the recorded current-time curve. The quantity due to the residual current which was measured separately with the same procedure was subtracted. The values thus obtained naturally included the electricity that was consumed in the reduction of the substance which had been produced as a result of the electro-reduction of tropolone followed by the chemical conversion, because the substance is easily reduced at the potential where tropolone is reduced. Moreover, the concentration of tropolone after a certain period of the electrolysis could not be determined accurately from the polarogram, because of the occurrence of the ill-defined reduction wave whose potentials are close to those of the reduction wave of tropolone. On account of those two unavoidable causes of error, the electricity required for the reduction of the unit concentration of tropolone could not be determined accurately in the usual way.

From the careful observation of the current-voltage curves obtained after the controlled potential electrolysis, it was found that the rate of formation of the new reducible substance was not extremely great. This indicates that a shorter period of electrolysis is more favorable for determining the quantity of electricity required for the reduction of tropolone itself. In an extreme case, at a zero time, only tropolone must be reduced. In the present investigation, therefore, the following procedure was employed. The controlled potential electrolyses were carried out in varied electrolysis periods with the solutions in which the initial concentration of tropolone and the pH were the same. The quantity of electricity was obtained from each current-time curve recorded. With the same solution as was subjected to the electrolysis the limiting current was measured, before and immediately after the electrolysis, with the dropping mercury electrode at potential where tropolone gives the diffusion current. The apparent amount of tropolone which was reduced in the given period was calculated from the difference of the limiting current by assuming the limiting current obtained immediately after the electrolysis to be caused by tropolone only. The apparent number of electrons required in the reduction was calculated by dividing the quantity of electricity with the product of the Faraday constant and the apparent molar amount of tropolone reduced in the period. The apparent numbers of electrons thus obtained for various electrolysis periods were found to have the tendency to decrease with the decrease of the electrolysis period. Thus, the true value of n could be obtained by plotting the apparent value of n against the electrolysis period and by extrapolating the curve to a zero time. An example of such a plotting is given in Fig. 5. The values

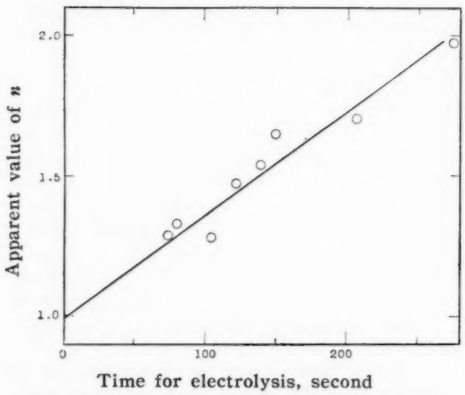


Fig. 5. Plot of apparent number of electrons involved in the reduction of tropolone against electrolysis period, measured at pH 5.35 at 20°C. The initial concentration of tropolone was 3.53×10^{-4} M.

of n obtained are given in the third column of Table III. Using the revised Ilkovič equation[7], the diffusion coefficients of tropolone were calculated from the limiting currents obtained at various pH's, which are given in the fourth column of Table III.

TABLE III
NUMBER OF ELECTRONS REQUIRED IN THE REDUCTION OF TROPOLONE AND DIFFUSION COEFFICIENT AT 25°C

pH	Wave used in the calculation	n	D, cm²/sec.
3.0	1st wave	1.0	9.0×10^{-6}
5.0	〃	1.0	7.6×10^{-6}
8.0		2.0	5.0×10^{-6}

Discussion

The diffusion coefficient of organic compounds can be evaluated with the aid of Oeholm's law[8],

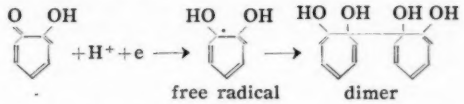
$$M^{1/2}D \fallingdotseq 7 \quad (\text{at } 20^\circ\text{C})$$

where M is molecular weight and D, the diffusion coefficient expressed in cm²/day. The value calculated for tropolone is 0.63 cm²/day, i. e., 7.4×10^{-6} cm²/sec. The diffusion coefficient of benzoic acid which is the isomer of tropolone was observed to be 9.2×10^{-6} cm²/sec. at 25°C[9] and is calculated to be 8.7×10^{-6} cm²/sec. at 25°C from the conductance data[10,11]. Comparing with those referred values, the diffusion coefficients of tropolone obtained in this study (Table III) seem to be reasonable.

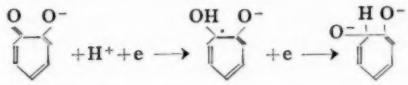
Tropolone molecules dissociate to form anions in an alkaline medium, the dissociation constant being 1.2×10^{-7} at 25°C[12]. Since the degree of interaction between the anion and the solvent molecules would be greater than that between undissociated tropolone and solvent molecules, the diffusion coefficient of the former is expected to be smaller than that of the latter, and, therefore, a smaller coefficient is expected in an alkaline medium. The experimental results given in Table III coincide with the above expectation.

As already indicated, one anodic wave and two cathodic waves were observed on the polarogram which was recorded with the solution subjected to the controlled potential electrolysis. The first wave which was anodic decreased with the lapse of time and the second which was cathodic increased. This showed that an oxidizable substance which is produced upon the electro-reduction of tropolone to give the anodic wave is unstable and converted gradually into a reducible substance which gives a cathodic wave at potentials more positive than tropolone.

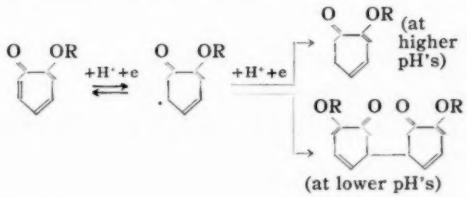
The mechanism of the electro-reduction of tropolones has been inferred from that of benzaldehyde[2,3]. Neish and Müller[3] gave the following to the first wave in the acid region:



The double wave in the intermediate pH region was assumed to appear according to the mechanism[3],



Recently Šantavý et al.[5] proposed the following processes for the reduction of tropolone and its ethers:



One-electron reduction followed by the dimerization was assumed at lower pH's, while two-electron reduction was assumed at higher pH's. In both cases the reduction of tropone nucleus was assumed.

In this study, the number of electrons involved in the electro-reduction of tropolone was determined from the coulometric measurement at constant potential. One-electron reduction at lower pH's and two-electron reduction at higher pH's were confirmed. This supports partly the reduction mechanism of tropolone previously proposed. However, the observation of current-voltage curves which were obtained with the solution preliminarily subjected to the controlled potential electrolysis strongly suggests that the chemical

7) M. von Stackelberg, *Z. Elektrochem.*, **57**, 338 (1953); etc.
8) L. W. Oeholm, *Z. physik. Chem.*, **70**, 378 (1910).
9) H. Freundlich and D. Krüger. *Biochem. Z.*, **205**, 186 (1929).
10) F. G. Brockman and M. Kilpatrick, *J. Am. Chem. Soc.*, **56**, 1483 (1934).
11) B. Saxton and H. F. Meiner, ibid., **56**, 1918 (1934).
12) N. Yui, *Sci. Repts. Tohoku Univ. First Ser.*, **40**, 102 (1956).

processes following on the electro-reduction are not so simple as has been proposed. The half-life period of the substance which formed upon the controlled potential electrolysis and gave the anodic wave would not indicate this substance to be the free radical described above, but a secondary substance produced therefrom. It is also unlikely that the dimer which Šantavý et al. assumed in their mechanism corresponds to the substance which gives the anodic wave, because the dimer seems not to be easily oxidizable. However, the difficulty in preparation of the substance because of chemical conversion, at present, seems to make it impossible to identify the substance. It would be desirable to carry out more elaborate studies for the precise explanation of the reduction mechnism.

Summary

The current-voltage curves of tropolone were recorded at the dropping mercury electrode in buffer solutions of various pH's containing no borate. One reduction wave was observed in the pH region of 2.2 to 4.0, two waves with nearly equal heights, in the pH region of 5 to 6 and one wave, in the solution of pH higher than 8. All waves except the second in the intermediate pH region were found to shift to negative potentials with the increase of pH of the solution by approximately 70 mV./pH. The number of electrons involved in the reduction was determined by the controlled potential coulometric method. One-electron reduction was confirmed for the first wave at lower pH's, two-electron reduction for the two-step waves at the intermediate pH region and two-electron reduction for the wave at higher pH's. The current-voltage curves which were recorded with the solution preliminarily subjected to the controlled potential electrolysis gave two new waves; one is anodic and the other, cathodic. The former decreased with time and the latter increased. This indicates that the reduction product of tropolone is unstable and changed gradually to the substance which gives the reduction wave. The diffusion coefficients of tropolone were calculated from the limiting current using the revised Ilkovič equation.

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Dipole Moments and Nuclear Quadrupole Coupling Constants of Alkyl Halides

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The relation between nuclear quadrupole coupling constants of molecules and the nature of chemical bonds has been discussed by Townes and Dailey[1]. Miyagawa[2] pointed out a parallelism between the nuclear quadrupole coupling constant and the apparent C—Cl bond moment of various chlorides.

It has been known that the dipole moment of alkyl halide increases with the increasing number of carbon atoms. The data are listed in Table I. This fact has hitherto been interpreted by an inductive effect[3], which, however, has not always given a satisfactory interpretation to it. At the same time, the values of nuclear quadrupole coupling constants of these molecules in the solid state decrease zigzag with the increasing number of carbon

1) C. H. Townes and B. P. Dailey, *J. Chem. Phys.*, **17**, 782 (1949).
2) I. Miyagawa, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **75**, 1061 (1954).
3) K. Higashi, *J. Chem. Soc. Japan* (*Nippon Kagaku Kaisi*). **57**, 1006 (1936); L. G. Groves and S. Sugden, *J. Chem. Soc.*, **1937**, 158.

TABLE I

DIPOLE MOMENTS* OF ALKYL HALIDE (DEBYE UNITS)

CH_3Cl	1.869[a]	CH_3Br	1.797[a]	CH_3I	1.647[a]
C_2H_5Cl	1.98[b]	C_2H_5Br	1.99[c]	C_2H_5I	1.93[c]
	2.00[c]		2.01[d]		1.87[d]
n-C_3H_7Cl	2.04[e]	n-C_3H_7Br	2.01[c]	n-C_3H_7I	1.97[c]
			2.15[d]		2.01[d]
n-C_4H_9Cl	2.11[d]	n-C_4H_9Br	2.15[d]	n-C_4H_9I	2.08[d]
	2.04[f]				
tert-C_4H_9Cl	2.13[g]				

* The observed values in the gaseous state.

a) W. Gordy, W. V. Smith and R. F. Trambarulo, "Microwave Spectroscopy", John Wiley and Sons, Inc., New York, N. Y., (1953), p. 361.

b) J. C. Jelatis, *Tech. Rep.*, **7**, O. N. R. Contract N5 ori—78 T. O. I. Lab. Ins. Res., Mass. Inst. Tech. (1947).

c) P. C. Mahanti, *Phil. Mag.*, (7) **20**, 274 (1935).

d) L. G. Groves and S. Sugden, *J. Chem. Soc.*, **1937**, 158.

e) R. Sanger, O. Steiger and K. Gachter, *Helv. Phys. Acta*, **5**, 200 (1932).

f) C. P. Smyth and K. B. McAlpine, *J. Chem. Phys.*, **3**, 347 (1935).

g) R. H. Wiswall and C. P. Smyth, ibid., **9**, 356 (1941).

TABLE II

NUCLEAR QUADRUPOLE COUPLING CONSTANTS OF ALKYL HALIDE (Mc)

Alkyl chloride (^{35}Cl)[a]		Alkyl bromide (^{79}Br)[b]		Alkyl iodide (^{127}I)[b]	
CH_3Cl	68.40	CH_3Br	528.9	CH_3I	1753
C_2H_5Cl	66.0	C_2H_5Br	497.0	C_2H_5I	1647
n-C_3H_7Cl	66.4	n-C_3H_7Br	503.0	n-C_3H_7I	1672
n-C_4H_9Cl	—	n-C_4H_9Br	499.9	n-C_4H_9I	1660
tert-C_4H_9Cl	62.3				

a) See foot-note a) appendix under Table I.

b) S. Kojima, K. Tsukada, S. Ogawa and A. Shimauchi, *J. Chem. Phys.*, **21**, 1415(1953).

atoms as shown in Table II. To throw light on these facts, the correlation among the electronic properties of the carbon-halogen bonds in alkyl halide molecules and the nuclear quadrupole coupling constants and the dipole moments of the molcules has been studied. A valence bond theory is developed to get the dependences of dipole moments and of nuclear quadrupole coupling constants on the ionicity of the carbon-halogen bond. The method used here is similar to that developed by Baughan, Evans and Polanyi[4].

Calculation and Results

The bonding wave function for a C—X (X=halogen) bond of an alkyl halide molecule can be constructed as follows:

$$\psi_{bCX} = (2+2S^2_{CX}+4\lambda S_{CX}+\lambda^2)^{-1/2} [\psi_X(1)\psi_C(2)+\psi_X(2)\psi_C(1) +\lambda\psi_X(1)\psi_X(2)] \quad (1)$$

where

$$\psi_X = a\psi_{ns}+b\psi_{np}$$
$$\psi_C = 1/2\psi_{2s}+\sqrt{3}/2\,\psi_{2p} \quad (2)$$

n=3, 4 and 5 for Cl, Br and I, respectively, and S_{CX} is the overlap integral defined by

$$S_{CX} = \int \psi_X(1)\psi_C(1)d\tau_{(1)}$$

The non-bonding hybrid orbital of the halogen atom is given by

$$\psi_{nb} = c\psi_{ns}+d\psi_{np}$$

From the orthogonality and normalization conditions for ψ_X and ψ_{nb}, we have

$$\left.\begin{aligned} a^2+b^2 &= 1 \\ c^2+d^2 &= 1 \\ ac+bd &= 0 \end{aligned}\right\} \quad (3)$$

The C—H bonds in methyl halide molecules are assumed to be pure covalent bonds, so that the wave functions for the bonds can be described as

$$\psi_{bCH} = (2+2S^2_{CH})^{-1/2} \times [\psi_C(1)\psi_H(2)+\psi_C(2)\psi_H(1)] \quad (4)$$

4) E. C. Baughan, M. G. Evans and M. Polanyi, *Trans. Faraday Soc.*, **37**, 377 (1941).

where

$$S_{CH} = \int \psi_C(1)\psi_H(1)d\tau_{(1)}$$

ψ_H is the 1s orbital of hydrogen atom, and ψ_C is assumed to be the tetrahedral hybrid orbital given by Eq. 2.

On the assumption of the orthogonality relationship between ψ_{bCX} and ψ_{bCH} the dipole moment of methyl halide is given by

$$\vec{\mu} = 3\vec{\mu}_{CH} + \vec{\mu}_{bCX} + \vec{\mu}_{nb}$$

where

$$\vec{\mu}_{CH} = e(2\overline{Z}_{CH} - R_{CH})$$
$$\vec{\mu}_{bCX} = e(2\overline{Z}_{CX} - R_{CX})$$
$$\vec{\mu}_{nb} = 2e\overline{Z}_{nb}$$

e is the electronic charge; R_{CH} and R_{CX} are the C—H and the C—X bond distances, respectively; the z-axis is taken along each bond direction with the carbon atom at the origin; $\overline{Z}_{CH}$, $\overline{Z}_{CX}$ and $\overline{Z}_{nb}$ are the average values of the z coordinates of the bonding electrons for the C—H and C—X bonds, and the non-bonding electrons of the halogen atom, respectively. The explicit expressions for Z_{CH}, Z_{CX} and Z_{nb} are well known, so that they are not described here.

A nuclear quadrupole coupling constant is usually expressed by eQq, where e is the electronic charge, Q is the nuclear quadrupole moment and q is the electric field gradient at the nucleus of the coupled atom with reference to the charge symmetric axis z, that is

$$q = e\sum_i \int \psi_i^*[(3\cos^2\theta_i - 1)/r_i^3]\psi_i d\tau_i$$

where the subscript i refers to the i-th electron, ψ_i is the wave function of the i-th one, r_i is the distance from the nucleus of the coupled atom, and θ_i is the angle between the r direction and the z axis. In the present work, only the valence electrons of the halogen atom are taken into account; the contributions from the others in the molecule are ignored, because the electrons on other atoms are too far away and the inner-shell clouds of the halogen atom have the symmetry to make the contribution to q insignificant. In this approximation, we obtain

$$q = 2q_{bCX} + 2q_{npx} + 2q_{npy} + 2q_{nb} \qquad (5)$$

where

$$q_\varphi = e\int \psi^*[(3\cos^2\theta - 1)/r^3]\psi d\tau$$

Now since q equals to naught for a completely filled p-shell, it can be easily be seen that

$$q_{npx} = q_{npy} = -\frac{1}{2}q_{npz} \qquad (6)$$

In consideration of Eqs. 3, 5 and 6, q is expressed as

$$q = [-b^2(1+2S^2_{CX}+2\lambda S_{CX})q_{npz} + 2(S_{CX}+\lambda) \times q_{CX} - q_C]/(1+S^2_{CX}+2\lambda S_{CX}+\lambda^2/2)$$

where

$$q_{CX} = e\int \psi_C[(3\cos^2\theta - 1)/r^3]\psi_X d\tau$$

In this case, q_C is very small compared with other terms, so the halogen quadrupole coupling constant may be written as

$$eQq = [-b^2(1+2S^2_{CX}+2\lambda S_{CX})eQq_{npz} + 2(S_{CX}+\lambda)eQq_{CX}]/(1+S^2_{CX} + 2\lambda S_{CX}+\lambda^2/2)$$

The atomic orbitals used in the calculation are the following hydrogen-like ones in which the screening constants were determined by Pauling and Sherman[5]: Cl($Z_{3s}=8.3$, $Z_{3p}=6.5$), Br($Z_{4s}=11.3$, $Z_{4p}=8.8$), I($Z_{5s}=14.5$, $Z_{5p}=11.7$), C($Z_{2s}=3.96$, $Z_{2p}=3.09$), H($Z_{1s}=1.00$). The bond distances are taken to be $R_{CH}=1.10$ Å, $R_{CCl}=1.78$ Å, $R_{CBr}=1.94$ Å, and $R_{CI}=2.14$ Å throughout the series of these alkyl halides.

The q_{npz} values calculated by use of these atomic orbitals, are shown in Table III. The results are in poor agreement with the accurate values. This means that such a theoretical procedure does not give satisfactory results because of the inaccuracy of the wave functions, especially in the part closer to the nucleus. In this work, therefore, the values of eQq_{npz} are taken from the experimental quadrupole coupling constants of atomic halogen;

TABLE III

	q_{np_z} values	
	$q_{calc.}$ (10^{15}e.s.u.)	$q_{obs.}$*(10^{15}e.s.u.)
$q^{Cl}_{3p_z}$	6.06	9.59
$q^{Br}_{4p_z}$	6.34	15.9
$q^{I}_{5p_z}$	7.63	~45

* These values were obtained by using the experimental quadrupole coupling constants[6] of atomic halogen and the quadrupole moments[a] of the halogen nucleus.

a) See foot-note a) appendix under Table I.

5) L. Pauling and J. Sherman, *Z. Krist.*, **81**, 1 (1932).

$eQq^{Cl}_{3p_z} = 109.74$Mc,[6] $eQq^{Br}_{4p_z} = -769.62$Mc[6] and $eQq^{I}_{5p_z} = 2292.44$Mc,[6] respectively.

The exchange term eQq_{CX} is evaluated by means of Löwdin's approximation[7] in which eQq_C is disregarded. In this procedure, μ and eQq are represented as the functions of λ and b^2, so that these values are determined by the experimental values of μ and eQq for methyl halide. For this purpose, the experimental values* for these gaseous molecules ($eQq_{CH_3Cl} = -75.3$Mc[3], $eQq_{CH_3Br} = 577.3$Mc[3] and $eQq_{CH_3I} = -1934$Mc[3]) are used. The values of λ and b^2 so obtained are given in Table IV.

TABLE IV

	CH_3Cl	CH_3Br	CH_3I
λ	1.0209	0.9134	0.6056
b^2	0.9749	0.9661	0.9456
$\mu_{bCX}(C^+X^-)$ (a.u.)	1.0321	0.7544	0.3107
μ_{nb} (a.u.)	0.6035	0.8516	1.2359

The determination of λ for the R—X bonds in alkyl halides (R=ethyl, *n*-propyl, *n*-butyl, *tert*-butyl) other than methyl halide is made by using the following process.

We obtain the following relation for the R—X bond from Eq. 1

$$\lambda^2/(2+2S^2_{CX}) = R_{ic}/(H_{ii}-E) \qquad (7)$$

where R_{ic} is the ionic-convalent resonance energy for the R—X bond, H_{ii} is the energy of the pure ionic structure R^+X^-, and E is the dissociation energy of the R—X bond.

Based on the assumption that the value of the factor b^2 is the same as that in methyl halide in the series of R-X bonds, the same value of overlap integral S_{CX} can be used for all R—X bonds. Thus, from Eq. 7, the value of λ can be determined if the values for R_{ic}, H_{ii} and E are known. The method is similar to that of Baughan, Evans and Polanyi[4]. They made an interesting application of the theory of ionic-covalent resonance to their correlation of changes in dipole moment and to bond energy along the series of alkyl derivatives R—X, in which R is methyl, ethyl, *n*-propyl or *tert*-butyl. They drew attention to the following trends along these series.

(1) The R—H bond energy decreases markedly.

(2) The R—X (X≠H) bond energy decreases less rapidly than the R—H bond energy.

(3) The dipole moment of the C—I bond increases parallel to the decrease in the R—I bond energy.

The data for the bond energy E(R—X) are shown in Table V. The interpretation has been given that the decrease in E(R—H) was attributed solely to the increasing resonance energy in alkyl radicals R_r, because the C—H bond was considered to be approximately a pure covalent bond. Hence

$$E(R—H) = E(CH_3—H) - R_r \qquad (8)$$

TABLE V

E(R-X) values*

R	E(C-H)[a]	E(C-Cl)[b]	E(C-Br)[a]	E(C-I)[b]
methyl	102.5	80.5	68.5	54.0
ethyl	97.5	79.4	67.0	52.0
n-propyl	95.0	77.4	64.5	50.0
n-butyl	94.0	—	63.5	49.0
tert-butyl	86.0	74.7	—	—

* All the E values are given in kcal. mole^{-1}.

a) E. T. Butler and M. Polanyi, *Trans. Faraday Soc.*, **39**, 19 (1943).

b) J. S. Roberts and H. A. Skinner, ibid., **45**, 339 (1959).

In the case of R—X bonds (X≠H), the ionic-covalent resonance energy in the R—X molecule increases along the series, due to the increased stability of the R^+X^- ion pair. Accordingly, there is the radical resonance energy R_r, which, as with R—H bonds, tends to decrease the bond energy, and there is an increase in ionic-covalent resonance energy R_{ic}, in the molecules, tending to increase the bonding energy. That is

$$E(R—X) = E(CH_3—X) - R_r + \Delta R_{ic} \qquad (9)$$

where

$$\Delta R_{ic} = R_{ic}(R—X) - R_{ic}(CH_3—X) \qquad (10)$$

The E(R—X) values in Table V are used in the present calculation and the R_r values are obtained by Eq. 8. By use of Eqs. 9 and 10, the R_{ic}(R—X) values can be evaluated, when we know the $R_{ic}(CH_3—X)$ values. These values are obtained from Eq. 7, using the λ, S_{CX} and $H_{ii}(CH_3—X)$ values. The $H_{ii}(CH_3—X)$ values are evaluated by using the Born-Meyer formula[8]

$$H_{ii} = e^2[-1/R_{CX} + \rho/R_i^2 \times \exp\{(R_i - R_{CX})/\rho\}] + I_C - E_X$$

6) H. Robinson, H. G. Dehmelt and W. Gordy, *J. Chem. Phys.*, **22**, 511 (1954).

7) P. O. Löwdin, ibid., **21**, 374 (1953).

* The dipole moment values used here are taken from those in Table I.

8) M. Born and J. E. Meyer, *Z. Physik.*, **75**, 1 (1932); E. Warhurst, *Trans. Faraday Soc.*, **45**, 461 (1949).

TABLE VI

Molecules	λ	μ_{bCCl} (a.u.)	$\Delta\mu_{bCCl}$ (D)	$\Delta\mu_{obs.}$* (D)	$\lvert eQq \rvert$ (Mc)	$\Delta eQq_{calc.}$ (Mc)	$\Delta eQq_{obs.}$ (Mc)
CH_3Cl	1.021	1.032	0	0	75.3	0	0
C_2H_5Cl	1.076	1.067	0.09	0.11 0.13	73.7	1.6	2.4
n-C_3H_7Cl	1.098	1.081	0.12	0.17	73.0	2.3	2.0
t-C_4H_9Cl	1.205	1.146	0.29	0.26	70.1	5.2	6.1

Molecules	λ	μ_{bCBr} (a.u.)	$\Delta\mu_{bCBr}$ (D)	$\Delta\mu_{obs.}$* (D)	$\lvert eQq \rvert$ (Mc)	$\Delta eQq_{calc.}$ (Mc)	$\Delta eQq_{obs.}$ (Mc)
CH_3Br	0.913	0.754	0	0	577.3	0	0
C_2H_5Br	0.971	0.797	0.11	0.19 0.21	564.7	12.6	31.9
n-C_3H_7Br	0.990	0.811	0.14	0.19 0.33	560.5	16.8	25.9
n-C_4H_9Br	0.998	0.817	0.16	0.33	558.8	18.5	29.0

Molecules	λ	μ_{bCI} (a.u.)	$\Delta\mu_{bCI}$ (D)	$\Delta\mu_{obs.}$* (D)	$\lvert eQq \rvert$ (Mc)	$\Delta eQq_{calc.}$ (Mc)	$\Delta eQq_{obs.}$ (Mc)
CH_3I	0.606	0.311	0	0	1934	0	0
C_2H_5I	0.666	0.365	0.14	0.22 0.28	1889	45	106
n-C_3H_7I	0.686	0.382	0.18	0.36 0.32	1875	59	81
n-C_4H_9I	0.692	0.387	0.19	0.43	1870	64	93

* These values are obtained from the data in Tables I and II.

where e is the electronic charge, ρ is given the value $\rho=0.345$ Å,[8] R_i is the equilibrium distance of R^+X^- ion pair, I_C is the ionization potential of carbon atom and E_X is the electron affinity of the halogen atom. The values of the quantities employed here are R_i(C—Cl)=2.17 Å[9], R_i(C—Br)=2.30 Å[9], R_i(C—I)=2.48 Å[9], I_C=11.42 eV[10], E_{Cl}=3.82 eV[10], E_{Br}=3.69 eV[10] and E_I=3.55 eV[10].

The H_{ii} values for R^+X^- ion pair are evaluated from $H_{ii}(CH_3—X)$ according to the following assumption by Baughan, Evans and Polanyi.

$$H_{ii}(R—X) \approx H_{ii}(CH_3—X) - R_r$$

Thus, using Eq. 7, we can calculate the λ values for the R—X bonds. The values of λ obtained and those of μ_{bCX} and eQq consequently obtained are shown in Table VI.

The variations of dipole moments and quadrupole coupling constants along the series of these molecules are interesting for us, so the quantities $\Delta\mu$, $\Delta\mu_{bCX}$ and ΔeQq defined as follows are introduced for comparisons between the observed values and the calculated ones.

$$\Delta\mu = \mu(R—X) - \mu(CH_3—X)$$

$$\Delta\mu_{bCX} = \mu_{bCX}(R-X) - \mu_{bCX}(CH_3—X)$$

$$\Delta eQq = eQq(R-X) - eQq(CH_3—X)$$

These values are listed in Table VI.

Discussion and Conclusion

In this calculation, the exchange terms eQq_{CX} are evaluated by Löwdin's approximation. Both Mulliken's approximation[11] and the neglect of the exchange terms have not always been sufficient for determining of b^2 and λ for all methyl halides. Usually these terms[1] have been evaluated as about a few percent or less of eQq_{np_z}. The values of these terms calculated here, however, are 19 percent of eQq_{3p_z} for the C—Cl bond, 14 percent of eQq_{4p_z} for the C—Br bond and 9 percent of eQq_{5p_z} for the C—I bond, respectively.

The b^2 values obtained here are greater than the values[1] estimated hitherto for Cl,

9) E. C. Baughan and M. Polanyi, ibid., **37**, 648 (1941).
10) H. O. Pritchard and H. A. Skinner, *Chem. Revs.*, **55**, 745 (1955).
11) R. S. Mulliken, *J. chim. phys.*, **46**, 500, 521 (1949).

less than those[1] for bromine and iodine though their accurate values have not yet been known. These greater values indicate the small amount of *s* character in the halogen bonding orbitals. It should be noted, however, that the so-called atomic dipole is considerably great, as seen in Table IV.

The magnitude of μ of alkyl halide depends to a great extent on those of μ_{bCX} and μ_{nb}, though it is not determined by them alone. It is seen from the results in Table VI that the variations of the C—X bond moments $\Delta\mu_{bCX}$ give a considerable contribution to those of molecule $\Delta\mu$. Furthermore, these results can be improved by taking account of the increasing *s* character in the halogen bonding orbitals along the series of these molecules, which means the elimination of the assumption that b^2 is constant along the series of these molecules, because it results in the increasing values of $\Delta(\mu_{bCX}+\mu_{nb})$* and of ΔeQq. They show that the variations of dipole moments along the series of these molecules should be interpreted by those of μ_{bCX} or $(\mu_{bCX}+\mu_{nb})$ instead of by an inductive effect.

The variations of dipole moments $\Delta\mu$ of the corresponding alkyl halides listed in Table VI increase in the sequence R—Cl, R—Br and R—I. This fact is not interpreted by an inductive effect. The present results suggest a qualitative explanation for this.

From these results, we can expect the values of eQq to decrease smoothly with the increasing carbon number. On the other hand, these values in the solid state fall zigzag as described above. This correlation is similar to the relations between the boiling points and the melting points of these molecules. It may be, therefore, reasonable that the zigzag of the eQq values in the solid state comes from the same reason as the one on which the irregularity between the melting points and the number of carbon is based.

In view of the present calculations, it seems highly probable that the eQq values of these molecules in the gaseous state will decrease smoothly with the increasing carbon number, though those in the solid state decrease zigzag with the increasing carbon number and that the ionic character of the C—X bonds in alkyl halide molecules increase with the increasing carbon number.

The author wishes to thank Professor K. Kozima for his kind criticism and Dr. J. Higuchi for his helpful advice and discussion.

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* The quantity is given the same definition as $\Delta\mu_{bCX}$ and so on.

Dielectric Contribution to Miscibility in the Ternary System 1-Propanol-Water-Toluene

By K. M. SOMASUNDARAM and C. V. SURYANARAYANA

(Received May 13, 1958)

When a third component miscible with each of the two liquids is added, to two layers of either immiscible or partially miscible liquids, the added component distributes between the two layers. Progressive additions of the third component results in a corresponding variation of the physico-chemical properties in the two layers till at the miscibility point they tend to reach a limit at which the interphase just disappears, forming a homogeneous system. Just at the point of disappearance of the interphase we may expect a quantitative relationship between the value of dielectric constant of the solution and those of the three pure components. The ternary of system 1-propanol, water and toluene has been studied from the above point of view. Incidentally the physico-chemical properties of different percentage compositions of the two binary systems 1-propanol-water and 1-propanol-toluene were also studied.

Experimental

Preparation of Solutions.—In clean dry 100 ml. Pyrex conical flasks different ratios of the two immiscible components, water and toluene, of the ternary system under study were taken by weight in such a way that the variation was progressive in the range of 10 to 90%. The third component, 1-propanol was added at a constant temperature of 35°C from a burette as in a usual titration and shaken thoroughly after each addition till a perceptible cloudiness appeared with no distinction between the two layers. Just a little more of the component from the burette was added cautiously in drops just till the cloudiness disappeared. Now the conical flask was weighed with its contents. From this, the weight of the added 1-propanol could be easily computed. From the weights of the individual components, the mole fractions of the constituents in the ternary mixture could be computed.

Measurement of Dielectric Constant, Density and Viscosity.—For measuring the dielectric constant of the homogeneous solution "Dekameter type DKO3" of Wissenschaftlich-Technische Werkstätten was used at a fixed frequency of 1.8 Mc/sec. with Hoppler's ultra thermostat. The homogeneous solution was put into the cell, allowed to attain the temperature of 35°C and then measurement was made.

Viscosity was measured as usual with an Ostwald viscometer using Gallenkamp's glass-walled thermostat b-12210. Density was determined using the density bottle, whose volume was determined by weighing it filled with water and dividing the weight of the water by the density at 35°C, which was looked up in Lange's[1] "Hand Book of Chemistry."

Purification of the Components.—E. Merck's Purest 1-propanol[2] was dried with anhydrous potassium carbonate and distilled. The fraction boiling between 97~98°C was collected and further dried with magnesium activated with iodine. The product was then distilled with exclusion of moisture and the distillate boiling at 97.2°C was collected and preserved in a Pyrex bottle. Baker's analysed toluene meeting the American Chemical Society specifications was dried over anhydrous calcium chloride, filtered and distilled. The distillate boiling at 110.6°C was collected and preserved in a Pyrex bottle. Conductivity water was distilled once more in a Pyrex outfit and this was preserved free from carbon dioxide and used. A dielectric constant check was made now and then. This water was used throughout the investigation.

Results obtained are detailed in the following tables.

Discussion

The equation for the molar polarization of a mixture

$$P_{1,2\cdots k}=\frac{\varepsilon_{1,2,\cdots k}-1}{\varepsilon_{1,2,\cdots k}+2}\frac{M_1x_1+M_2x_2+\cdots M_kx_k}{\rho_{1,2\cdots k}} \quad (1)$$

holds strictly for solutions of zero volume of mixing. Therefore, in normal practice, apart from many other factors that influence the dielectric constant of a solution the occurrence of the density factor in equation 1 limits its rigorous application.

Van Arkel and Snoek[3] adopt the equation

$$L_{1,2}=L_1v_1+L_2v_2 \quad (2)$$

where v is the volume concentration and L the volume polarization. Sugden[4] found

1) N. A. Lange, "Hand Book of Chemistry", Hand Book Publishers, Inc., Sandusky, Ohio (1949) p. 1385
2) A. I. Vogel, "Practical Organic Chemistry", Longmans, London, 2nd ed., (1951), p. 168.
3) A. E. van Arkel and J. L. Snoek, *Trans. Faraday Soc.*, **30**, 710 (1934).
4) S. Sugden, *Nature*, **133**, 415 (1934).

that when P_2 is plotted against volume polarization for solutions of benzo nitrile, nitrobenzene and chlorobenzene in a number of non-polar solvents, all the points for each substance lie near a straight line through the entire range of solutions to the pure liquid. For the change in polarization of the solute in concentrated solution Sugden[5] found a very simple relation ship

$$_cP_2 = A - B\frac{\varepsilon_c - 1}{\varepsilon_c + 2} \quad (3)$$

where A and B are constants. From these considerations it is clear that volume polarization seems to be more fundamental than molar polarization.

Böttcher[6] derives the equation

$$\frac{\varepsilon - 1}{\varepsilon + 2} = \sum_k \varphi_k \cdot \frac{\varepsilon_k - 1}{\varepsilon_k + 2} \quad (4)$$

which is the same as

$$L_{1,2\cdots k} = L_1V_1 + L_2V_2 \cdots L_kV_k \quad (5)$$

where $\frac{\varepsilon - 1}{\varepsilon + 2} = L_{1,2\cdots k}$ = volume polarization of the mixture, $\varphi_k = V_k$ = volume fraction of the component k, and $\frac{\varepsilon_k - 1}{\varepsilon_k + 2} = L_k$ = volume polarization of the component k.

In equation 5 V_k is the ratio of the volume v_k of component k to the total volume $v_1 + v_2 \cdots v_k$ in the mixture. Let $n_1, n_2 \cdots n_k$ be the actual number of molecules contained in $v_1, v_2 \cdots v_k$ cc. respectively. Therefore the volume fraction V_k equals effectually $n_k / \sum n_k$ which is the same as the mole fraction x_k. The total volume polarization $L_{1,2\cdots k}$ of the mixture as measured will be due to the sum of total contribution of $\sum n_k$ molecules. Hence we may write,

$$\sum_k L_k x_k = L_{1,2\cdots k} \quad (6)$$

Equation 6 is clearly independent of the volume of mixing. Volume polarization L is a dimensionless function and depends only on the number of molecules present in unit volume and their polarisabilities.

Data obtained in the above investigations have been used to compare equation 6 with the well known equation

$$\sum_k P_k x_k = P_{1,2\cdots k} \quad (7)$$

Table I records the data obtained for the ternary system. Table II brings out a comparison of the equations 6 and 7. It is quite clear that percentage deviation from the mean value is almost negligible with equation 6, whereas deviations are considerable with equation 7.

Akerlöf[7] determined the dielectric constant of mixtures of 1-propanol and water at 25°C. Table III gives the data on density, viscosity and dielectric constant of mixtures of 1-propanol and water at 35°C as determined in this laboratory.

Plots of molar polarization and volume polarization against mole percent of 1-propanol (not given) show a linear variation. The plot of dielectric constant against mole percent of alcohol (not given) falls off rapidly with increasing mole percent of alcohol. The viscosity curve (not given) shows a maximum at about 28 mole percent of 1-propanol. The last column of Table III shows that at about 26 mole percent of 1-propanol, the volume contraction is maximum. If from viscosity and volume contraction data we have to conclude any molecular combination involving solute-solvent interaction we must expect with Hassel and Uhl[8] anomalously high molecular polarization about 28 mole percent of 1-propanol which is obviously not true here. It is also known[9,10] that the effect of association on the dielectric constant depends on the sizes and shapes of the participating dipoles. As a result of a greater electrostatic moment of a polymerized molecule than the simple one the dielectric constant of the solvent will be greater in its associated state. Deassociation in some solutions is indicated by the lowering of the dielectric constant of that component in solution. Conversely when dipole-dipole interaction occurs, the forces holding the electric charge will be weakened as a result of probable increase of the electrostatic moment of the molecule and the dielectric constant of a component becomes greater in solutions than in its pure state. In this context merely from the viscosity peak, it is rather unsafe particularly in the absence of absorption and Raman spectra, to conclude the existence of a compound of whatever composition between 1-propanol-water. Further investigations are required to solve this

5) S. Sugden, *Trans. Faraday Soc.*, **30**, 720 (1934).
6) C. J. F. Böttcher, "Theory of Electric Polarisation", Elsevier, Amsterdam, (1952), p. 203.
7) G. Akerlöf, *J. Am. Chem. Soc.*, **54**, 4125 (1932).
8) O. Hassel and A. H. Uhl, *Naturwissenschaften*, **18**, 247 (1930).
9) R. N. Kerr, *J. Chem. Soc.*, **1926**, 2796.
10) J. W. Williams and I. J. Krchma, *J. Am. Chem. Soc.*, **48**, 1886 (1926).

TABLE I
(1-Propanol-water-toluene).

Mole fraction			Density	Viscosity in centipoises	Dielectric constant	Volume contraction in ml. per 1000 ml. of solution
1-Propanol x_1	Water x_2	Toluene x_3				
0.5042	0.2793	0.2166	0.8326	0.8804	12.70	4.861
0.4888	0.3351	0.1761	0.8355	0.9553	14.49	8.557
0.4645	0.3996	0.1359	0.8399	1.0360	16.50	8.971
0.4138	0.4976	0.0886	0.8487	1.1840	20.35	10.720
0.3657	0.5778	0.0565	0.8588	1.3160	25.00	12.650
0.3191	0.6400	0.0409	0.8694	1.3910	28.48	14.700
0.2754	0.6928	0.0318	0.8794	1.4740	32.00	15.770
0.2197	0.7603	0.0200	0.8936	1.5620	37.53	10.840
0.1389	0.8501	0.0110	0.9169	1.7000	46.80	7.316

TABLE II

$\sum_{k=1,2,3} P_k x_k$	$P_{1,2,3}$	% deviation from the mean value	$\sum_{k=1,2,3} L_k x_k$	$L_{1,2,3}$	% deviation from the mean value
44.885	52.85	8.149	0.7672	0.7960	1.843
43.500	50.56	7.506	0.7952	0.8181	1.419
41.702	47.51	6.510	0.8239	0.8377	0.831
38.529	42.83	5.286	0.8600	0.8658	0.336
35.738	38.91	4.251	0.8857	0.8888	0.175
33.257	35.76	3.659	0.9011	0.9018	0.042
31.033	33.15	3.301	0.9115	0.9118	0.014
28.198	29.73	2.644	0.9251	0.9243	0.043
24.111	25.27	2.347	0.9395	0.9387	0.043

TABLE III
(1-Propanol-water)

Mole fraction		Density	Viscosity in centipoises	Dielectric constant	Volume contraction in ml. per 1000 ml. of solution
1-Propanol x_1	Water x_2				
—	1.0000	0.9941	0.723	74.94	—
0.0243	0.9757	0.9815	0.849	69.75	6.69
0.0599	0.9400	0.9661	1.038	64.37	14.91
0.1044	0.8956	0.9454	1.232	58.50	17.46
0.1392	0.8608	0.9305	1.348	51.38	19.75
0.2072	0.7928	0.9063	1.472	43.30	19.36
0.2629	0.7371	0.8902	1.519	38.75	20.16
0.3383	0.6617	0.8722	1.523	34.00	18.68
0.4273	0.5727	0.8553	1.494	29.71	17.44
0.5839	0.4161	0.8326	1.406	25.00	13.08
0.8250	0.1750	0.8085	1.290	20.95	8.40
1.0000	—	0.7918	1.614	18.90	—

question.

Data of dielectric constant, density and viscosity of mixtures of 1-propanol and toluene, are given in table V. Table VI shows that both the equations 6 and 7 fail with the system.

Plots of molar polarization and volume polarization (not given) against percentage composition of 1-propanol show a continuous increase with a tendency for a maximum in the former about 75 mole percent of the alcohol. The dielectric constant and viscosity (curves not given) vary with mole percent of 1-propanol in a similar way, increasing rapidly and continuously with increase of alcohol.

Several other binary systems worked recently in this laboratory show that both equations 6 and 7 are valid in polar-polar binary mixtures and definitely fail in the case of polar-nonpolar mixtures. It is rather difficult to explain this fact.

TABLE IV

$\sum_{k=1,2} P_k x_k$	$P_{1,2}$	% deviation from the mean value	$\sum_{k=1,2} L_k x_k$	$L_{1,2}$	% deviation from the mean value
—	17.42	—	—	0.9610	—
18.568	18.59	0.0592	0.9582	0.9583	0.0052
20.254	20.28	0.0641	0.9543	0.9548	0.0262
22.387	22.53	0.3111	0.9500	0.9504	0.0211
24.041	24.23	0.3914	0.9463	0.9441	0.1163
27.280	27.55	0.4922	0.9392	0.9337	0.2936
29.940	30.27	0.5480	0.9337	0.9264	0.3924
35.520	33.88	0.5342	0.9254	0.9166	0.4889
37.754	38.11	0.4953	0.9164	0.9055	0.5955
45.207	45.48	0.3010	0.9000	0.8890	0.6151
56.670	56.69	0.0176	0.8747	0.8694	0.3039
—	65.01	—	—	0.8566	—

TABLE V

(1-Propanol-toluene)

Mole fraction		Density	Viscosity in centipoises	Dielectric constant	Volume contraction in ml. per 1000 ml. of solution
1-Propanol x_1	Toluene x_2				
—	1.000	0.8528	0.5474	2.34	—
0.1148	0.8852	0.8459	0.5495	2.82	0.825
0.2642	0.7358	0.8393	0.5691	4.00	−2.248
0.3860	0.6140	0.8335	0.6002	5.09	−0.224
0.5022	0.4978	0.8272	0.6466	6.90	−0.223
0.6123	0.3877	0.8215	0.7062	9.25	1.045
0.7121	0.2879	0.8157	0.7902	11.50	2.396
0.8095	0.1905	0.8097	0.8888	14.05	2.840
0.9353	0.0647	0.8005	1.0880	17.43	3.186
1.0000	—	0.7918	1.6140	18.90	—

TABLE VI

$\sum_{k=1\ 2} P_k x_k$	$P_{1,2}$	% deviation from the mean value	$\sum_{k=1,2} L_k x_k$	$L_{1,2}$	% deviation from the mean value
—	33.42	—	—	0.3092	—
37.056	39.51	3.205	0.37217	0.3777	0.738
41.760	49.83	8.918	0.45380	0.5000	4.848
45.610	55.25	9.557	0.52060	0.5772	5.156
49.270	60.94	10.590	0.58400	0.6630	6.336
52.760	64.74	10.200	0.64450	0.7335	6.604
55.935	66.13	8.345	0.69926	0.7778	5.318
58.986	66.48	5.973	0.75231	0.8130	3.877
62.962	65.63	2.075	0.82111	0.8455	1.462
—	65.01	—	—	0.8566	—

Tables I, III and V show that in all cases there is involved some volume contraction. Equation 6 holds excellently in the ternary system. Equation 6 cannot be claimed, therefore, to have set right the error due to volume of mixing inherent in equation 1. On the other hand as observed recently with several other ternary systems in this laboratory, equation 6 may be considered to give tentatively a quantitative condition involving a dielectric contribution of the components to miscibility. In other words, just at the point wherein two phases disappear to give a homogeneous system, equation 6 seems to apply excellently.

Summary

In place of the familiar equation $\sum_k P_k x_k = P_{1,2\cdots k}$, which does not hold good in many cases, we have formulated alternatively

the equation $\sum_k L_k x_k = L_{1,2\cdots k}$ where L is volume polarization. This equation has been applied to the ternary system 1-propanol-water and toluene just at the point of miscibility, when to a given ratio of toluene and water addition of an optimum amount of 1-propanol results in the disappearance of the two phases. Also the new equation has been applied to two fully miscible binary systems 1-propanol-water and 1-propanol-toluene. It has been concluded that the new equation gives a condition concerning the dielectric contribution of the components to miscibility in the ternary system.

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Stress Relaxation of Non-electrolytic Thermo-reversible Gels.— Concentration Dependence of Distribution Function of Relaxation Times. Polyvinyl Alcohol-Glycerin-Water Gels*

By Kiyoshi ARAKAWA

(Received January 28, 1958)

According to the reversibility of sol-gel transition with temperature, gels are classified into two groups, thermo-reversible and thermo-irreversible. As typical thermo-reversible gels we have agar, gelatine, etc. But most of these are polyelectrolytes, rheological properties of which are complicated by charge effects. In the present work the rheological property of non-electrolytic thermo-reversible gels was examined. A system consisting of polyvinyl alcohol, glycerin, and water was used as an example of such a gel. The elastic behavior of the gel has been studied previously by N. Hirai[1], but the distribution function of relaxation times was not obtained for this gel. Such a function has been determined from stress relaxation of the gel and discussed here briefly.

Experimental

Apparatus.—A chainomatic balance relaxometer shown in Fig. 1 was used to measure Young's modulus of a sample under constant strain. The relaxometer is of similar type with those used by S. L. Dart and E. Guth[2] for the study of stress relaxation in cork, and recently by several workers in studies of polymers[2].

In this relaxometer a sample is strained by compression betweeen two plates, the upper of which is attached to the horizontal bar of chainomatic balance. The lower plate is connected with a screw gauge. The stress produced by the constant strain is balanced with a chain, whose unfixed end is moved by a balancing motor. The

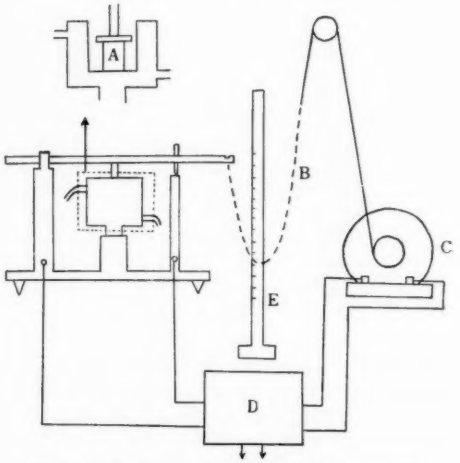

Fig. 1. Schematic diagram of chainomatic balance relaxometer.
A: specimen
B: chain
C: balancing motor
D: relay circuit
E: scale

* Presented in part at the 10th Annual Meeting of the Chemical Society of Japan held in Tokyo, April 1957.

1) N. Hirai, *J. Chem. Soc. Japan, Pure Chem. Sec.*, (*Nippon Kagaku Zassi*), **74**, 235 (1953); ibid., **74**, 259 (1953).

2) S. L. Dart and E. Guth, *J. Appl. Phys.*, **17**, 314 (1946); H. Fujita and K. Ninomiya, *J. Polymer Sci.*, **24**, 233 (1957); This Bulletin, **26**, 24 (1953); K. Ogino, the 9th Annual Meeting of Chem. Soc. Japan (1956).

motor is driven through a relay circuit which is connected with a point contact attached to the bar. From the suspended length of the chain the stress is calculated and Young's modulus of the sample under constant strain can be obtained. The temperature of the sample is controlled by water circulated from a thermostat into a double jacket which contains the sample.

Materials.—In the present study a commercial polyvinyl alcohol was used throughout. The amount of residual ester groups in the sample was 2% by mole. The intrinsic viscosity $[\eta]_{20}$ of this unfractionated sample was 0.27, which was determined by means of a capillary viscometer of Ostwald type. The viscosity average molecular weight M_V was 1.1×10^4 according to the formula by H. Staudinger and H. Warth[3].* As solvent a mixture of glycerin and water was used with a mixing ratio of 4:1 by volume.

The preparation of testing specimens was as follows. A definite quantity of polyvinyl alcohol powder was mixed with glycerin and water in a conical beaker. Being covered with liquid paraffin, the powder was dissolved slowly by heating in an oil bath kept at 125±5°C. In adjusting of the mixing ratio 4:1 of glycerin and water the presence of adsorbed water in polyvinyl alcohol powder, the amount of which was determined before each preparation, was taken into account. After the polyvinyl alcohol powder was dissolved completely, the solution was poured into a mould and kept at about 90°C for about thirty minutes. It was then cooled down slowly to about 50°C in four hours, and was aged at room temperature. Gels of three different concentrations, 8.3%, 9.9% and 12.1 % by weight were prepared.

Results and Discussion

Stress Relaxation Data.—The data of stress relaxation of gels of three different concentrations are plotted in the form $\log(298E_r/T)$ vs. $\log t$, where E_r is Young's relaxation modulus and t time, in Fig. 2 a, b and c**.

These data are reduced to 25°C by multipling E_r by a factor $298/T$. The multiplication by this factor is often subjected to objection, but in the present work the stress relaxation experiment was made in rubbery region mainly, and therefore this reduction is thought to be reasonable.

According to the time temperature superposition principle[4] a master relaxation curve at 25°C for each concentration may be composed by shifting these relaxation curves along the $\log t$ axis. The master relaxation curves for each concentration, thus obtained, are plotted in Fig. 3.

The amounts of the shift along the $\log t$ axis at various temperatures, $\log a_T$, are plotted against $1/T$ in Fig. 4. They give a nearly straight line. The apparent

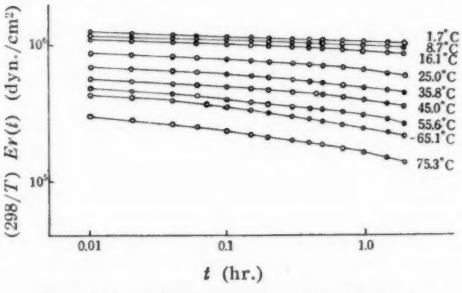

Fig. 2 (a). Reduced Young's relaxation modulus $\frac{298}{T}E_r(t)$ of 8.3% gel.

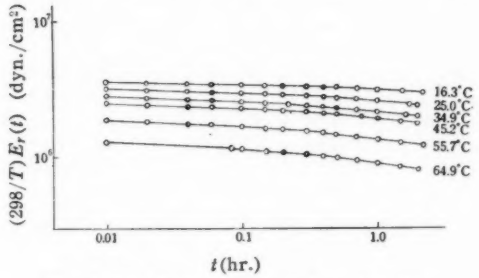

Fig. 2 (b) Reduced Young's relaxation modulus $\frac{298}{T}Er(t)$ of 9.9% gel.

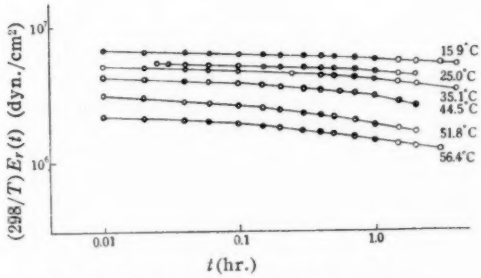

Fig. 2 (c). Reduced Young's relaxation modulus $\frac{298}{T}E_r(t)$ of 12.1% gel.

3) H. Staudinger and H. Warth, *J. Prakt. Chem.*, **155**, 261 (1944).

* In application of this formula, the effect of the residual ester groups was ignored.

4) A. V. Tobolsky and R. D. Andrews, *J. Chem. Phys.*, **11**, 125 (1943); R. D. Andrews, N. Hofman-Bang and A. V. Tobolsky, *J. Polymer Sci.*, **3**, 669 (1948); J. D. Ferry, *J. Am. Chem. Soc.*, **72**, 3746 (1950); F. Schwarzl and A. J. Staverman, *J. Appl. Phys.*, **23**, 838 (1952); C. A. Dahlquist and M. R. Hatfield, *J. Colloid Sci.*, **7**, 253 (1952).

** Compressions applied in the present experiment were 3% in 12.1% and 9.9% gels, and 5% in 8.3% gel. In this range of compressions the linear relation between stress and strain was almost maintained.

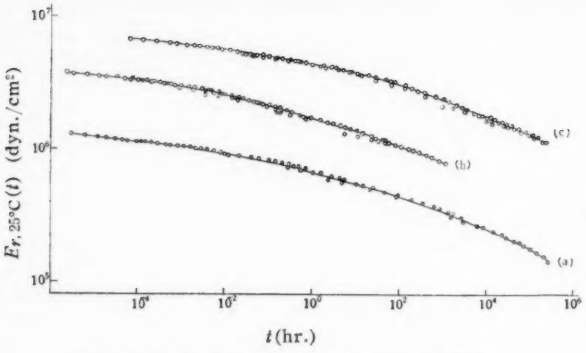

Fig. 3. Master relaxation curves at 25°C.
(a) 8.3%, (b) 9.9%, (c) 12.1%

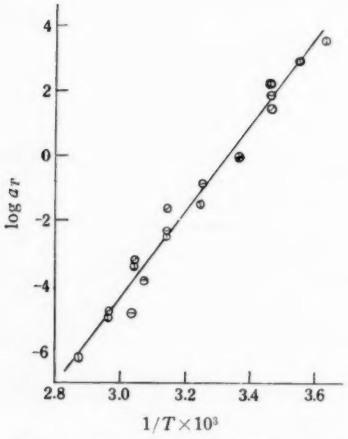

Fig. 4. Log a_T versus $1/T$ relation.

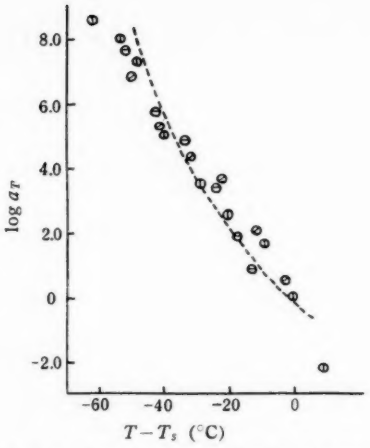

Fig. 5. Log a_T versus $T-T_s$ relation.
◑ 8.3%, ◴ 9.9%, ⊖ 12.1%
T_s: 338°K for 8.3%
340°K for 9.9%
342°K for 12.1%
------ equation of WLF form[5]
$C_1=8.86$, $C_2=101.6$

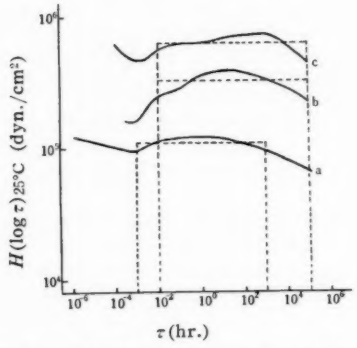

Fig. 6. Distribution functions of relaxation times.
(a) 8.3%, (b) 9.9%, (c) 12.1%

activation energy H_a, defined as $R\partial\ln a_T/\partial(1/T)$ is 60 kcal./mole, which is nearly kept constant over the temperature- and concentration-range in the present work. As for the temperature dependence of the factor a_T a function on the log a_T vs. $T-T_s$ relation has been proposed recently by M. L. Williams, R. F. Landel and J. D. Ferry[5], where T_s is a reference temperature. The coincidence between the values of a_T obtained in this work and the function is fairly good in the range of $T-T_s$, $-40\sim0$°C, but outside this range they deviate from the curve, as seen in Fig. 5.

Distribution Functions of Relaxation Times.—A distribution function of relaxation times $H(\log\tau)$ may be determined from a master curve, using the following first order approximation[6]

$$H(\log\tau)=-2.303[E_r\mathrm{d}\log E_r/\mathrm{d}\log t]_{t=\tau} \quad (1)$$

It was found that the difference between the first and the second order approximation was small enough in the calculated values to be ignored. Therefore the former was used throughout.

Logarithms of $H(\log\tau)$, thus obtained, are plotted against $\log\tau$ in Fig. 6.

5) M. L. Williams, R. F. Landel and J. D. Ferry, *J. Am. Chem. Soc.*, **77**, 3701 (1955).

6) R. D. Andrews, *Ind. Eng. Chem.*, **44**, 707 (1952); F. Schwarzl, *Physica*, **17**, 830, 865 (1951).

TABLE I

VALUES OF PARAMETERS OF BOX-TYPE DISTRIBUTION

Concn. (wt. %)	τ_1 (hr.)	τ_2 (hr.)	$\overline{H}\times10^{-5}$ (dyn./cm^2)	τ_m (hr.)	$H_m\times10^{-5}$ (dyn./cm^2)
8.3	10^{-3}	10^{3}	1.1	10^{0}	1.2
9.9	10^{-2}	10^{5}	3.3	$10^{1.5}$	4.0
12.1	10^{-2}	10^{5}	6.3	10^{3}	7.1

Concentration Dependence of Master Relaxation Curve and Relaxation Spectrum.—It is seen in Fig. 3 that the master curve shifts upwards and to the longer time side with increase of concentration. But, when seen in details, the concentration dependence of the master curve is fairly complicated. Therefore, it is impossible to reduce the curves for different concentrations to a composite curve, as carried out by T.W. DeWitt et al.[7] on the decalin solution of polyisobutylene and previously by J. D. Ferry et al.[7]

Also, in Fig. 6 we can see that the relaxation spectrum exhibits a fairly complicated nature on the concentration dependence. Simple approximations of box type for each spectrum are shown by dotted lines. Three parameters characterizing a box type distribution, two abscissae τ_1 and τ_2 ($\tau_1<\tau_2$), corresponding to two vertical sides, and the height $\overline{H}$ of the box are tabulated in Table I, together with the maximum value of $H(\log\tau)$, H_m and the abscissa τ_m at the maximum point. The value of $\overline{H}$ is taken to be an average value of $H(\log\tau)$ between τ_1 and τ_2. As seen in Fig. 6 and Table I, the maximum point in the region shifts upwards and to the longer time side with increase of concentration. The relation between $\overline{H}$ and concentration seems to be complicated. To draw any conclusion on this relation, further studies are required. Also it is evident that the shift of the spectrum due to the change of concentration is not uniform over the region covered in the present study. Concerning the shift along the $\log\tau$ axis, the minimum point which appears in the short time region nearly retains its position, while the maximum point shifts to the longer-time side, as the concentration increases. From this non-uniformity of the shift it is tentatively supposed that the relaxation mechanism in this region consists of two or more groups of mechanisms, which exhibit the different dependence on the concentration of polyvinyl alcohol.

7) T. W. DeWitt, H. Markovitz, F. J. Padden and L. J. Zapas, *J. Colloid Sci.*, **10**, 174 (1955); J. D. Ferry, *J. Am. Chem. Soc.*, **72**, 3746 (1950); W. M. Sawyer and J. D. Ferry, ibid., **72**, 5030 (1950).

Summary

From a stress relaxation experiment by means of a chainomatic balance relaxometer the stress relaxation curves were obtained for the gel of polyvinyl alcohol-glycerin-water system, which was taken as an example of non-electrolytic thermo-reversible gel. Gels of 8.3%, 9.9% and 12.1% were used. The temperature of the samples was changed between about 2°C and 75°C. Master relaxation curves were obtained for each concentration over the range of time, $10^{-4}\sim10^{5}$ hours, using the time temperature superposition principle. According to Alfrey's approximation, relaxation spectra were calculated.

The following results were obtained.

1) The master relaxation curves exhibit a fairly complicated nature on concentration dependence.

2) The $\log a_T$ vs. $T-T_s$ relation obeys approximately the formula by Williams, Landel and Ferry in the range of $T-T_s$, $-40\sim0$°C, but outside the range deviates fairly from it.

3) The apparent activation energy is 60 kcal./mole, which is nearly kept constant over the temperature- and concentration-range in the present work.

4) The shift of the relaxation spectrum due to the change of concentration is not uniform over the region of box type. The maximum point in that region shifts upwards and to the longer-time side with increase of concentration.

The author wishes to express his gratitude to Mr. K. Ogino of Tokyo University for his helpful advice in designing the relaxometer, and Dr. T. Nakagawa of Tokyo University for his encouragement throughout this work.

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On the Bromination Reaction of Tropone and its Bromine Compounds[1]

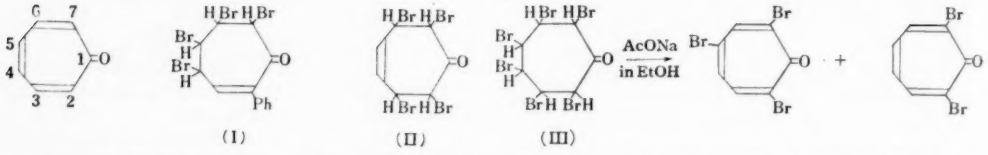
By Toshio MUKAI

(Received April 21, 1958)

On bromination, tropone is not directly subject to replacement with bromine but intermediately forms a bromine addition product and subsequently forms the true bromine-substitution product by its dehydrobromination and debromination[2,3]. This fact gives a new observation regarding the aromaticity of tropone and is one of the experimental facts that indicate highly unsaturated characteristic of tropone compared to tropolone. It has been noticed, however, that tropones show different modes in the bromine addition according to their structures. For example, bromination of 2-phenyltropone chiefly gave a tetrabromide (I) and indicated that the C_2-C_3 double bond of I was fairly resistant to further bromination owing to the steric hindrance by the phenyl group at C_2 position[3]. Tropone itself was also brominated to form the tetrabromide (II) but its dehydrobromination gave 2,7-dibromotropone and this indicated that the C_4-C_5 double bond of II was resistant to bromine addition[2]. In addition, bromination of 2-bromotropone also afforded 2,7-dibromotropone via the addition product, and this dibromotropone was not further brominated even on application of an excess of bromine in chloroform or acetic acid with heating[2]. These facts also indicated that the C_4-C_5 double bond in tropone ring was inactive to bromine addition or substitution. In 1951, Brown[4] suggested that the C_2 (or C_7) position on the tropone ring will be most easily submitted to electrophilic substitution from his calculation by the molecular orbital method, and this is rather interesting when the above facts are considered.

In the present paper, further bromination reaction of tropone and its various bromine-substituted derivatives is reviewed and examinations are made on whether the double bond at C_4-C_5 of II is entirely inactive to bromine addition. Also what effect the bromine atoms present in the tropone ring have on this addition reaction is described.

Tropone invariably affords 2,7-dibromotropone, on causing an excess of bromine to react under heating and it is known to give the tetrabromide (II) when cold[2]. Therefore, different reaction conditions were adopted. Tropone was dissolved in glacial acetic acid or chloroform, an excess of bromine was added, and the mixture was allowed to stand for a long period. The oily addition product obtained by this reaction differs from II and its analytical value agrees with that for a hexabromide. Its dehydrobromination with sodium acetate was found to give chiefly 2,4,7-tribromotropone, though accompanied with 2,7-dibromotropone. It has become clear through this experiment that the addition product obtained here has the structure of III and that the double bond at C_4-C_5 submits to bromine addition under the above conditions. The ultraviolet spectrum of the addition product III is shown in Fig. 1 and there is no marked absorption maximum. Its infrared spectrum[5] has no



(I) (II) (III)

1) This paper was read before the 9th Annual Meeting of the Chemial Society of Japan held in Kyoto on April 5, 1956.

2) T. Nozoe, T. Mukai and K. Takase, *Science Repts. Tohoku Univ.*, **1**, **39**, 164 (1956).

3) T. Mukai, ibid., **1**, **38**, 281 (1954).

4) R. D. Brown, *J. Chem. Soc.*, **1951**, 2670.

5) Infrared spectrum was measured with Perkin-Elmer Model 21 double beam spectrophotometer by Mr. Yusaku Ikegami of the Chemical Research Institute of Non-aqueous Solutions, to whom the author's deep gratitude is hereby expressed.

marked absorption in the C=C region either. These facts support the conclusion that the addition compound has the saturated ketone structure of III. The absorption of C=O in III appears as two bands* at 1735 and 1706 cm^{-1}. Toda and Kitahara[6] have found that the equatorial

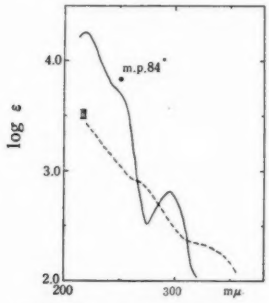


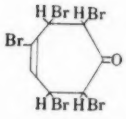
Fig. 1. U. V. absorption spectra of addition product III in cyclohexane and of compound melted at 84°C in methanol.

α-bromine atom of bromosuberones shifts its carbonyl band to the higher frequency by 17 cm^{-1} than that of suberone at 1701 cm^{-1}. The absorption at 1735 cm^{-1} is higher in frequency by 34 cm^{-1} than that of suberone, so this is thought to indicate the fact that the two α-bromine atoms of III are in equatorial to C=O.

The addition product III was dissolved in glacial acetic acid and refluxed or heated in an oil bath to 140~150°C without a solvent[7]. In this case, hydrogen bromide and bromine were evolved and in both cases, only 2,7-dibromotropone was obtained and not the 2,4,7-tribromo compound.

Similarly, when 2-bromotropone[8] and bromine in glacial acetic acid solution are allowed to stand over a long period of time, a pentabromo-compound is obtained. Dehydrobromination of this addition product by heating in ethanol, in the presence of sodium acetate, easily affords 2,4,7-tribromotropone, from which it is certain that this addition product has the structure shown by IV. It is known from this that the bromine addition occurs not at the C_2-C_3 double bond but at C_4-C_5 double bond. Decomposition of IV by heating in acetic acid results in evolution of hydrogen bromide and bromine, and 2,7-dibromotropone alone is obtained, while its decompostition by heating without a solvent chiefly gives 2,7-dibromotropone accompanied by a small amount of 2,4,7-tribromotropone. It should be noted that in these cases also elimination of free bromine has occurred at C_4-C_5 bond.

(IV)



(V)

Bromination of tropone and 2-bomotropone under heating affords 2,7-dibromotropone, as mentioned earlier, and there is a possibility that the dibromo compound is formed through the addition products, III and IV. In order to confirm this point, sodium acetate was added in this bromination but the 2,7-dibromotropone was invariably obtained and not the 2,4,7-tribromotropone. Therefore, it may be concluded that bromination with heating does not form addition products like III and IV.

Application of an excess of bromine to acetic acid solution of 4-bromotropone[9] results in direct substitution reaction and 2,4,7-tribromotropone is obtained, the bromine addition compounds being obtained with difficulty. In this case, the use of chloroform in place of acetic acid as a solvent affords pentabromo compound as the addition product and this addition product invariably forms 2,4,7-tribromotropone, either by heating in ethanol with sodium acetate or by heating without a solvent or in acetic acid solution. These facts support the structure of V for the addition compound. Decomposition of V in ethanol in the presence of sodium acetate gives, besides 2,4,7-tribromotropone, colorless crystal melting at 84°. This latter substance is stable and neutral and its ultraviolet and infrared spectra are shown respectively in Figs. 1 and 2. From this ultraviolet spectrum, it is seen that the substance no longer retains a tropone ring[10] but its structure remains unsolved.

* It may owe to the presence of two conformationally different isomers.

6) T. Toda. and Y. Kitahara, to be published. (From the dissertation of T. Toda, Tohoku University) cf. E. J. Corey, *J. Am. Chem. Soc.*, **75**, 2301 (1953).

7) These reaction conditions were taken in order to compare them with that used in the troponization (bromination-dehydrobromination) of suberone. cf. T. Nozoe, Y. Kitahara, T. Ando, S. Masamune and H. Abe, *Science Repts. Tohoku Univ.*, **1**, **36**, 166 (1952); *Proc. Japan Acad.*, **27**, 415 (1951).

8) T. Nozoe, S. Seto and S. Matsumura, *Proc. Japan Acad.*, **28**, 483 (1952).

9) T. Nozoe, T. Mukai, Y. Ikegami and T. Toda, *Chem. & Ind.*, **1955**, 66.

10) M. Tsuboi, This Bulletin, **25**, 369 (1952).

The infrared spectrum of V exhibits an absorption band of C=C at 1605 cm^{-1} and of C=O group at 1738 cm^{-1}. The latter band is 37 cm^{-1} higher in frequency than C=O band of suberone at 1701 cm^{-1} and this will indicate the fact that the two α-bromine atoms of V are located in the equatorial position[6].

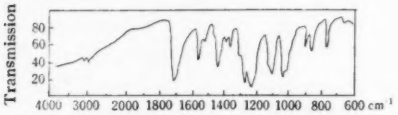
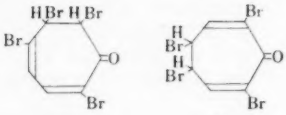


Fig. 2. I. R. absorption spectrum of compound melting at 84°C in KBr.

The behavior of 2,5-dibromotropone[9] toward bromination is similar to that of 4-bromotropone, that is, it forms 2,4,7-tribromotropone on standing with bromine in acetic acid solution at room temperature. However, the use of chloroform as a solvent also affords the tetrabromo-compound. This addition compound always forms 2,4,7-tribromotropone, either on dehydrobromination with sodium acetate or on heating alone or in acetic acid solution.



(VI) (VII)

From these facts, the structure represented by VI is given to this addition compound, and also, in this case the double bond with the bromine is found to be resistant to bromine addition. The infrared spectrum of VI exhibits absorptions at 1658 cm^{-1} for C=O and at 1613 cm^{-1} for C=C, and these values seem approximately as suitable as those for such a conjugated, unsaturated ketone. The bromine addition compound of tropones having bromine atom at C_4 or C_5 position, as in V and VI, tends to decompose and the ultraviolet spectra of V and VI in a polar solvent like methanol exhibit absorption similar to that of 2,4,7-tribromotropone resulting from the decomposition. It is assumed that the failure to obtain addition compounds from the bromination of 4-bromo- and 2,5-dibromotropone in acetic acid solution may be related to this instability.

When 2,7-dibromotropone[8] is allowed to stand with an excess of bromine in acetic acid solution at room temperature for a long period, a tetrabromo compound crystallizes out. Heating with sodium acetate in ethanol solution, affords 2,4,7-tribromotropone together with 2,7-dibromotropone. This proves that the structure of the addition compound is correctly represented by VII. The infrared spectrum of VII has absorptions for conjugated C=O and C=C respectively at 1656 and 1613 cm^{-1}. Heating VII alone or in acetic acid solution results in evolution of bromine and only 2,7-dibromotropone is formed and this also indicates that bromine is easily eliminated from C_4–C_5 bond.

The foregoing bromination reaction of tropones may be summarized as follows: 1) Bromine addition of tropone seems to proceed in a stepwise manner, the addition being facile up to two moles, but addition to the C_4–C_5 double bond requires a long period. This is thought to be due to the increased repulsion between the bromine atoms and between the carbonyl group and bromine atom by the formation of hexabromo compound (III) by the third mole of bromine. 2) Further addition of bromine to bromotropones does not occur in the double bond carrying the bromine atom and this is probably due to increased repulsion between bromine atoms by the formation of a geminated dibromo-structure[11]. 3) The bromine atoms attached to the C_4–C_5 positions tends to be easily liberated as bromine, while those bonded to C_2–C_3 and C_6–C_7 positions are liberated as hydrogen bromide. 4) It is difficult to derive tropone, 2-bromotropone, and 2,7-dibromotropone by bromination into 2,4,7-tribromotropone without going through the addition compounds.

The important point in connection with the foregoing bromination reaction of tropone is the troponization of suberone by the action of bromine[7]. This is one of the fundamental reactions in the preparation of tropones but, because the reaction is so complicated, its reaction mechanism has not been clarified as yet. Therefore, several polybromosuberones that form as intermediates during this reaction have been isolated and their troponization reaction has been examined[12]. The bromine addition compounds formed in the present series of experiments may also be regarded as allied substances of polybromo compounds of suberone, but

11) The addition compound having geminated two bromine atoms was obtained on the bromination of 2-phenyltropone. cf. Ref. 3.

12) Paper presented at the Local Meeting of Tohoku district of the Chemical Society of Japan held in Yamagata on June 28, 1954.

it should be noted that there is a fair difference between the compounds of these two series as to the position and configuration of the bromine atom. It is interesting that troponization of suberone by bromination affords 2,4,7-tribromo-, 2,5-dibromo-, and 4-bromotropones[9], and not 2-bromo- or 2,7-dibromotropones, while the bromine addition compounds obtained by the bromination of tropone invariably are 2-bromo-[2] and 2,7-dibromotropone rather than 2,4,7-tribromotropone.

Experimental[13]

Bromination of Tropone.—*a) In carbon tetrachloride solution.*—To a solution of 150 mg. of tropone dissolved in 3 cc. of carbon tetrachloride, 950 mg. (4.2 mol. equiv.) of bromine was added, by which reddish orange oil separated out and later crystallized. This mixture was allowed to stand in a sealed vessel at room temperature and the crystal again changed to an oil. After about 3 weeks, the mixture containing oil was dried in a vacuum desiccator over potassium hydroxide and paraffin to get constant weight, and 800 mg. of hexabromo compound (III) was obtained as yellowish orange syrup, which was submitted to elemental analyses and ultraviolet and infrared spectral measurements.

Anal. Found: C, 14.95; H, 1.25. Calcd. for $C_7H_6OBr_6$: C, 14.36; H, 1.02%. I. R. Spectrum: $\tilde{\nu}$ (C=O) 1735, 1706 cm^{-1}, (C=C) very weak band (without solvent).

b) In acetic acid solution.—A mixture of 100 mg. of tropone dissolved in 1.5 cc. of acetic acid and 950 mg. (6.3 mol. equiv.) of bromine was allowed to stand for 10 days at room temperature and treated as in a), from which 600 mg. of yellowish orange oil was obtained. The analytical values of this oil did not agree with those for the hexabromo compound (Found: C, 17.74; H, 1.83) but its decomposition with sodium acetate afforded 2,4,7-tribromotropone and thermal decomposition gave 2,7-dibromotropone, exactly as in the case of III (q. v.).

c) Bromination in the presence of sodium acetate under heating.—A mixture of 30 mg. of tropone, 230 mg. of bromine (5 mol. equiv.) and 70 mg. of sodium acetate in 1.5 cc. of acetic acid was heated for 3 hours on a water bath. At the end of this period, some bromine still remained. Acetic acid was removed and residual crystals were washed with ethanol and water, affording 60 mg. of pale greenish yellow crystals, m. p. 145~160°C. Recrystallization from ethanol-benzene mixture gave 40 mg. of crystals melting at 167~169°C, undepressed on admixture with 2,7-dibromotropone, m. p. 170°C.

Bromination of 2-Bromotropone.—*a) In acetic acid solution.*—To a solution of 150 mg. of 2-bromotropone dissolved in 2 cc. of acetic acid, 640 mg. (5 mol. equiv.) of bromine was added and the mixture was allowed to stand in a sealed vessel for one month at room temperature. Crystals began to separate out from the tenth day. The crystals were collected by filtration to give 320 mg. of colorless crystals, m. p. 115°C (decomp. with efferv.). Recrystallization from ethanol gave colorless needles IV, m. p. 135°C (decomp. with efferv.).

Anal. Found: C, 15.92; H, 1.09. Calcd. for $C_7H_5OBr_5$: C, 16.61; H, 1.00%. U.V. Spectrum: $\lambda_{max}^{cyclohexane}$ mμ (log ε): 280(4.35). I. R. Spectrum: $\tilde{\nu}$ (C=O, C=C) 1736, 1704, 1607 cm^{-1} (in KBr disk).

b) Bromination in the presence of sodium acetate under heating.—A mixture of 50 mg. of 2-bromotropone and 80 mg. of sodium acetate in 2 cc. of acetic acid containing 170 mg. (4 mol. equiv.) of bromine was heated on a water bath for 3 hours. Acetic acid was removed, and the residue was washed with water. Filtration afforded 70 mg. of pale brown crystals, m. p. 130~150°C. Washing with ethanol gave 40 mg. of crystals melting at 162~166°C, and recrystallization from ethanol-benzene mixture gave pale yellow plates, m. p. 169~170°C. This was found to be 2,7-dibromotropone by mixed fusion.

Bromination of 4-Bromotropone.—*a) In acetic acid solution.*—To a solution of 400 mg. of 4-bromotropone dissolved in 10 cc. of acetic acid, 1.73 g. (5 mol. equiv.) of bromine was added and the mixture was allowed to stand in a sealed vessel at room temperature. After about one month, some needle crystals crystallized out but the mixture was allowed to stand for a total of 3 months and the crystals were collected. The first crop of crystals was 450 mg. of pale yellow needles, m. p. 183°C. The mother liquor was allowed to stand over potassium hydroxide in a vacuum desiccator and a second crop of 15 mg. of crystals, m. p. 175~179°C, and a third crop of 15 mg. of crystals, m. p. 173~175°C, were obtained. All these crystals showed no depression of the melting point on admixture with 2,4,7-tribromotropone, m. p. 183°C. The final residual oil decomposed on heating, evolving bromine and afforded 90 mg. of 2,4,7-tribromotropone, m. p. 177~179°C.

b) In chloroform solution.—To a solution of 150 mg. of 4-bromotropone dissolved in 3 cc. of chloroform, 390 mg. (3 mol. equiv.) of bromine was added, and the mixture was allowed to stand in a sealed vessel for one month. This was then dried to constant weight over potassium hydroxide and paraffin in a vacuum desiccator and 410 mg. of reddish orange viscous oil V was obtained. This oil began to crystallize after a few days but showed such a tendency to decompose that the oil itself was submitted to analyses and spectral measurements.

Anal. Found: C, 16.54; H, 1.38. Calcd. for $C_7H_5OBr_3$: C, 16.61; H, 1.00%. I. R. Spectrum: $\tilde{\nu}$ (C=O, C=C) 1738, 1675, 1605 cm^{-1} (without solvent).

On washing with benzene, the crystals which melted at ca. 85°C with decomposition were obtained but after standing, they showed m. p. 125~140°C.

13) m.p. are uncorrected. The microanalyses were carried out by Mr. Shin-ichi Ohyama to whom the author's sincere acknowledgements are hereby extended.

Bromination of 2,5-Dibromotropone. — *a) In acetic acid solution.*—One gram (3 mol. equiv.) of bromine was added to a solution of 550 mg. of 2,5-dibromotropone dissolved in 6 cc. of acetic acid and the mixture was allowed to stand in a sealed vessel at room temperature. Needle crystals began to crystallize out after one month and the crystals were collected after a total of two months. The first crop of 100 mg. of pale yellow needle crystals, m. p. 183~184°C, were collected and the filtrate was dried over potassium hydroxide, affording a second crop of 220 mg. of crystals, m. p. 180~181°C. Both were identified with 2,4,7-tribromotropone by mixed fusion. The final mother liquor also began to crystallize but was dissolved in chloroform and passed through a short column of alumina. Removal of the solvent from the effluent afforded 220 mg. of crystals which melted at 80~120°C with decomposition. It probably contained some addition products but recrystallization from benzene gave only 50 mg. of 2,4,7-tribromotropone, m. p. 178~180°C, and the residue could not be purified further.

b) In chloroform solution.—To a solution of 280 mg. of 2,5-dibromotropone dissolved in chloroform, 510 mg. (3 mol. equiv.) of bromine was added and the mixture was allowed to stand in a sealed vessel for 2 weeks. This was dried to a constant weight over paraffin and potassium hydroxide in a vacuum desiccator to remove the chloroform and 460 mg. of reddish orange oil VI was obtained. This oil was submitted to analyses and spectral measurements as it had a tendency to undergo decompostition.

Anal. Found: C, 19.97; H, 1.16. Calcd. for $C_7H_4OBr_4$: C, 19.83; H, 0.95%. I. R. Spectrum: $\tilde{\nu}$ (C=O, C=C) 1658, 1613 cm^{-1}.

After about 2 days, the oil crystallized and melted at 60~65°C with decomposition, but recrystallization from ethanol afforded crystals melting at 165~173°C, and indicated that the majority had changed to 2,4,7-tribromotropone.

Bromination of 2,7-Dibromotropone. — *a) In acetic acid solution.*—Three hundred milligrams (3.3 mol. equiv.) of bromine was added to a solution of 150 mg. of 2,7-dibromotropone dissolved in 15 cc. of acetic acid and the mixture was allowed to stand for one month at room temperature. This mixture was dried over potassium hydroxide in a vacuum desiccator to give 200 mg. of pale yellow crystals, m. p. 155°C (decomp.), which was recrystallized from benzene and afforded pale yellow prisms VII, m. p. 156~157°C (decomp.).

Anal. Found: C, 20.45; H, 1.61. Calcd. for $C_7H_4OBr_4$: C, 19.83; H, 0.95%. U. V. Spectrum: $\lambda_{max}^{cyclohexane}$ $m\mu$ (log ε): 230(3.95), 295(3.73). I. R. Spectrum: $\tilde{\nu}$ (C=O, C=C) 1656, 1613 cm^{-1}. (in KBr disk).

In another experiment by the same procedure, some crystals other than VII were obtained, which partially melted at 105°C and melted with decomposition at 130°C (Anal. Found: C, 16.55; H, 0.94%).

b) Bromination under heating.—A solution of 70 mg. of 2,7-dibromotropone dissolved in 3 cc. of acetic acid containing 130 mg. (3 mol. equiv.) of bromine was sealed in a tube and heated in a boiling water bath for 6 hours. The mixture was dried over potassium hydroxide in a vacuum desiccator and 70 mg. of pale yellow plates, m. p. 167~169°C, were obtained. This was the recovered starting material.

c) Bromination in the presence of sodium acetate under heating.—A mixture of 50 mg. of 2,7-dibromotropone and 50 mg. of sodium acetate dissolved in 2 cc. of acetic acid containing 90 mg. (3 mol. equiv.) of bromine was heated for 3 hours. Treatment as above afforded 50 mg. of plae yellow crystals, m. p. 145~163°C, which on recrystallization from ethanol-benzene mixture gave 30 mg. of crystals melting at 168~169°C and 5mg. of those melting at 160~163°C. Both were found to be 2,7-dibromotropone by admixing.

Reaction of Various Bromine Addition Compounds with Sodium Acetate in Ethanol Solution. — *a) 2, 3, 4, 5, 6, 7-Hexabromocycloheptanone (III).*—A solution of 300 mg. of III and 200 mg. of sodium acetate dissolved in 5 cc. of ethanol was refluxed on a water bath for 30 minutes. The ethanol was evaporated off from the mixture and a small amount of water was added to the residual solution by which 100 mg. of pale yellow needles, m. p. 180~181°C, was obtained. The mixed melting point proved it to be 2,4,7-tribromotropone. This mother liquor, was diluted with water, extracted with chloroform and the extract was passed through a column of alumina. The alumina was eluted with ethanol and evaporation of ethanol from the effluent left 100 mg. of colorless crystals, m. p. 157~162°C, which showed no depression on admixture with 2,7-dibromotropone but the mixed melting point was depressed on fusion with 2,4,7-tribromotropone. Therefore, this was assumed roughly to be 2,7-dibromotropone.

b) 2, 4, 5, 6, 7-Pentabromo-2-cyclohepten-1-one (IV).—To a solution of 80 mg. of IV dissolved in 5 cc. of ethanol, 100 mg. of sodium acetate was added and the mixture was heated on a water bath for 50 minutes. Evaporation of ethanol left 30 mg. of pale yellow needles, m. p. 178~180°C, and the melting point was raised to 180~181°C on recrystallization from benzene, undepressed on admixture with 2,4,7-tribromotropone.

c) 2, 3, 4, 6, 7-Pentabromo-4-cyclohepten-1-one (V).—A mixture of 200 mg. of V and 100 mg. of sodium acetate in 4 cc. of ethanol was refluxed on a water bath for 30 minutes and thereafter treated as in a), from which 90 mg. of crystals, m. p. 110~155°C (sint. 80°C), was obtained. Recrystallization from ethanol afforded 30 mg. of pale yellow crystals, m. p. 173~175°C, whose mother liquor will be designated as (A). The melting point of these crystals was raised to 179~180°C by recrystallization from benzene and admixed melting point test identified them to be 2,4,7-tribromotropone.

Ethanol was evaporated off from the mother liquor (A), the residue was dissolved in chloroform, and the solution was passed through a short alumina column. Evaporation of chloroform

from the effluent gave 50 mg. of crystals melting at 70~80°C. The crystals were dissolved in carbon tetrachloride and again passed through a short alumina column. Crystals obtained from the effluent were recrystallized from ethanol and afforded 30 mg. of colorless plates, m. p. 83~84°C.

(*Anal.* Found: C, 33.59; H, 2.53%.)

d) 2,5,6,7-Tetrabromo-2,4-cycloheptadien-1-one (VI).—A solution of 130 mg. of oily VI and 70 mg. of sodium acetate dissolved in 2.5 cc. of ethanol was heated on a water bath for 30 minutes, water was added to the mixture, and crystals that separated out were collected by filtration to give 90 mg. of yellowish needles, m. p. 179~180°C, undepressed on admixing with 2,4,7-tribromotropone.

e) 2,4,5,7-Tetrabromo-2,6-cycloheptadien-1-one (VII).—A mixture of 100 mg. of VII and 100 mg. of sodium acetate in 2.5 cc. of ethanol was refluxed on a water bath for 30 minutes, ethanol was evaporated, and water was added to the residue by which 70 mg. of pale yellow silky crystals, m. p. 155~170°C, were obtained. Recrystallization from benzene first afforded 30 mg. of crystals melting at 182~183°C, identified by admixture with 2, 4, 7-tribromotropone. Concentration of this recrystallization mother liquor gave another crop of crystals, m. p. 160~173°C, which were dissolved in chloroform and passed through an alumina column. Elution with ethanol afforded 20 mg. of crystals, m. p. 169~170°C, showing no depression on admixture with 2,7-dibromotropone.

Thermal Decomposition of Various Bromine Addition Compounds in Acetic Acid Solution.—*a) 2, 3, 4, 5, 6, 7-Hexabromocycloheptanone (III).*—A solution of 120 mg. of III dissolved in 2 cc. of acetic acid was refluxed in an oil bath for 3 hours, during which the evolution of hydrogen bromide (blue coloration of Congo-red paper) and bromine (blue coloration of iodine-starch paper) was observed. Acetic acid was then evaporated from the solution, and the residue was washed with ethanol, affording 40 mg. of pale yellow crystals, m. p. 150~161°C. The crystals were dissolved in chloroform and the solution was passed through a short alumina column. Evaporation of chloroform from the effluent left crystals melting at 160~165°C, which were recrystallized from ethanol-benzene mixture, giving 25 mg. of pale yellow plates, m. p. 170~171°C, identified as 2,7-dibromotropone by mixed fusion.

b) 2, 4, 5, 6, 7-Pentabromo-2-cyclohepten-1-one (IV).—A solution of 80 mg. of IV dissolved in 2 cc. of acetic acid was refluxed on a water bath for 3 hours, acetic acid was evaporated, and the crystalline residue was washed with ethanol, affording 30 mg. of colorless plates, m. p. 169~170°C, undepressed on admixture with 2,7-dibromotropone.

c) 2, 3, 4, 6, 7-Pentabromo-4-cyclohepten-1-one (V).—A solution of 80 mg. of V dissolved in 2cc. of acetic acid was refluxed for 3 hours and thereafter treated as in b), affording 45 mg. of crystals melting at 125~140°C. Recrystallization from ethanol gave 20 mg. of pale yellow needles, m. p. 178~180°C, identified as 2, 4, 7-tribromotropone by mixed fusion.

Ethanol was evaporated from the recrystallization mother liquor, the residue was dissolved in chloroform, and passed through an alumina column. This effluent gave 10 mg. of pale yellow crystals, m. p. 120~135°C, which were not identified.

d) 2, 5, 6, 7-Tetrabromo-2, 4-cycloheptadien-1-one (VI).—A solution of 90 mg. of VI dissolved in 2 cc. of acetic acid was refluxed for 3 hours and thereafter treated as in b), affording 30 mg. of dark brownish crystals, m. p. 165~175°C. The crystals were dissolved in benzene and passed through an alumina column, and evaporation of the solvent left yellow needles, m. p. 172~176°C, undepressed on admixing with 2,4,7-tribromotropone.

e) 2,4,5,7-Tetrabromo-2,6-cycloheptadien-1-one (VII).—A solution of 60 mg. of VII dissolved in 2 cc. of acetic acid was refluxed in an oil bath for 3 hours and thereafter treated as in b), affording 40 mg. of pale yellow plates, m. p. 169~170°C, identified with 2,7-dibromotropone by mixed fusion.

Thermal Decomposition of Various Bromine Addition Compound.—*a) 2,3,4,5,6,7-Hexabromocycloheptanone (III).*—One hundred milligrams of III was placed in a small test tube and dipped in an oil bath at 145~150°C by which the compound melted and decomposed, with evolution of hydrogen bromide and bromine. The contents were heated at this temperature for 20 minutes, cooled, and the crystalline residue was washed out with ethanol, affording 40 mg. of dark brown crystals, m. p. 140~150°C. The crystals were dissolved in benzene, passed through a column of alumina, and the column was eluted with chloroform. The benzene eluate gave a few mg. of crystals, m. p. 140~150°C, and the chloroform effluent gave 25 mg. of colorless crystals, m. p. 169~170°C. Mixed fusion proved the latter to be 2,7-dibromotropone.

b) 2, 4, 5, 6, 7-Pentabromo-2-cyclohepten-1-one (IV).—One hundred milligrams of IV was heated and decomposed in a small test tube as in a) and 50 mg. of colorless crystals, m. p. 145~150°C, were obtained. Recrystallization from ethanol afforded colorless plates, m.p. 150~157°C. These crystals were dissolved in chloroform, passed through an alumina column, and eluted with ethanol. Chloroform effluent afforded a few mg. of colorless crystals, m. p. 170~172°C, and ethanol eluate afforded 30 mg. of colorless plates, m. p. 167~169°C. By mixed fusion, the former was identified with 2, 4, 7-tribromotropone and the latter with 2, 7-dibromotropone.

c) 2, 3, 4, 6, 7-Pentabromo-4-cyclohepten-1-one (V).—Thermal decomposition of 120 mg. of V in a small test tube as in a) afforded 60 mg. of pale brown crystals, m. p. 177~179°C, which were recrystallized from ethanol to pale yellow needles, m. p. 182~183°C and identified with 2, 4, 7-tribromotropone by admixture with an authentic sample.

d) 2, 5, 6, 7-Tetrabromo-2, 4-cycloheptadien-1-one (VI).—Thermal decomposition of 120 mg. of VI in a small test tube as in a) afforded 70 mg. of pale yellow crystals, m. p. 179~181°C, which showed no depression on admixing with 2, 4, 7-tribromotropone.

e) 2, 4, 5, 7-Tetrabromo-2, 6-cycloheptadien-1-one (VII).—Thirty milligrams of VII was placed in a small test tube and heated for 20 minutes in an oil bath at 160°C. After cooling, the crystalline residue was sublimed at 5 mm. Hg in a bath at 140°C and afforded 15 mg. of crystals melting at 163~166°C. Recrystallization from benzene raised the melting point to 167~169°C, undepressed on admixing with 2, 7-dibromotropone.

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Reaction of Troponoids and Organometallic Compounds. III[1,2]*. Synthesis and Reaction of 2,7- and 2,3-Diphenyltropone*[3]

By Toshio MUKAI

(Received April 21, 1958)

In the reaction of 2-phenyltropone and phenylmagnesium bromide, two compounds with the composition $C_{19}H_{14}O$, m. p. 87°C and 130°C, were obtained[4]. From their analytical values and ultraviolet and infrared spectra, one of these was assumed to be 2, 7-diphenyltropone but there still remained a doubt about this structure for the following reasons. The first is the fact that this reaction would be substitution of a ring hydrogen with phenyl, which would be very unlikely to occur. The second is that 2, 7-diphenyltropone is an unknown compound and it would probably have properties fairly different from tropones due to a hindered carbonyl group.

The present paper deals with the synthesis of 2, 7-diphenyltropone by an unequivocal process, establishing the structure of the foregoing products from the Grignard reaction, and at the same time, examinations are made on the properties of tropones substituted in the 2- and 7-positions[5].

2, 7-Diphenyltropone was synthesized by the reaction of 3-phenyltropolone and phenyllithium. Doering and Hisky[6] had already obtained 2-phenyltropone by the reaction of tropolone and phenyllithium. Later Haworth and Tinker[7] proved that although an abnormal substitution occurred in the Grignard reaction of 4-methyltropolone methyl ethers, the reaction of the copper complex of 4-methyltropolone and phenyllithium resulted in the normal substitution, affording 6-methyl-2-phenyltropone. Since the reaction of tropolones and phenyllithium results firstly in the formation of the lithium salt of tropolones, it may be assumed that it would result in the normal substitution, as in the case of the copper complex, to form 2-phenyltropones.

Actually, the reaction of 3-phenyltropolone and phenyllithium afforded two kinds of compound, one of m. p. 132°C (I) and the other of m. p. 147°C (II).

These were assumed to be the corresponding diphenyltropones from their analytical values and the infrared absorption

1) Part I: T. Nozoe, T. Mukai, J. Minegishi and T. Fujisawa, *Science Repts. Tohoku Univ.*, **1**, **37**, 388 (1953).
2) Part II: K. Kikuchi, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **77**, 1439 (1956).
3) This paper was read before the Local Meeting of Tohoku District of the Chem. Soc. Japan held in Hirosaki on September 27, 1956.
4) T. Nozoe, T. Mukai and others, unpublished work.
5) A number of 2,7-dibenzyltropones were synthesized, of which the chemical behavior has not been much described. N. J. Leonard, L. A. Miller and J. W. Berry, *J. Am. Chem. Soc.*, **79**, 1482 (1957).
6) W. von E. Doering and C. F. Hisky, ibid., **74**, 5688 (1952).
7) R. D. Haworth and P. B. Tinker, *J. Chem. Soc.* **1955**, 911.

spectra[8] shown in Fig. 1. As will be shown later, II was identified as 2,3-diphenyltropone by its conversion into 2,3-diphenylbenzoic acid. I was identified with one of the products, of m. p. 130°C, from the foregoing reaction of 2-phenyltropone and phenylmagnesium bromide as expected. From the course of these two reactions, it may be concluded that I is 2,7-diphenyltropone. It follows, therefore, that the reaction of tropolone and phenyllithium results in the normal substitution.

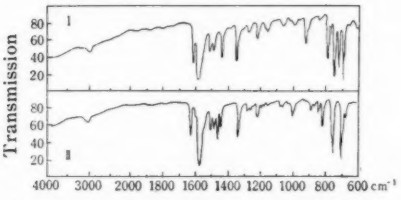


Fig. 1. I. R. absorption spectra of 2,7-diphenyl-(I) and 2,3-diphenyltropone (II) in KBr.

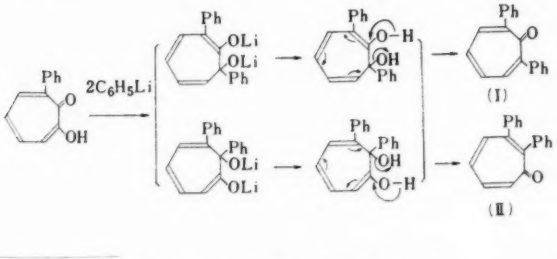
The reaction of tropolone methyl ether and methylmagnesium iodide does not give 2-methyltropone[9], but, since 2-alkyltropones are obtained by the reaction of tropolone and methyllithium[10] or *n*-butyllithium[6], it may be assumed that a lithium compound convenient for the formation of tropones is formed as an intermediate. This may be illustrated as shown below with the reaction of 3-phenyltropolone and phenyllithium as an example. This reaction is rather interesting when compared with the fact that a ketone and not a carbinol is formed in the reaction of free carboxylic acid and an organolithium compound[11].

The ultraviolet spectra of 2,3- (II) and 2,7-diphenyltropone (I) are shown in Fig. 2. The absorption of I is very similar to that of 2-phenyltropone[1,12] but is shifted slightly to a longer wave length region. On the contrary, the absorption of II is shifted to a much shorter wave length region than that of I and indicates that the phenyl groups in the 2- and 3-positions have a fair amount of steric strain.

Both I and II have weak basicity and do not form picrates. Passage of hydrogen chloride gas through the benzene solution of I or II only precipitates a part of the hydrochloride.

Since the two reactive sites, 2- and 7-positions, in I are blocked, the reactivity of 2,7-diphenyltropone is of interest. I is extremely stable to alkalis and amines, and it remains unchanged on heating with dilute alkali or *p*-toluidine in ethanol solution[1,13]. Heating with hydroxylamine or hydrazine fails to effect amination of the ring[1,13]. I does not react with ketonic reagents such as 2,4-dinitrophenylhydrazine[1,13]. These facts indicate that the substitution of 2- and 7-positions of the tropone ring with a bulky group like phenyl results in an extremely great increase in stability and the above reactions characteristic to tropones in general are not observed.

In contrast to the stability of I, 2,3-diphenyltropone (II) is labile to alkali and on heating with dilute alkali in ethanolic solution, it forms an acid substance as colorless crystals, m. p. 178°C. The ultraviolet spectrum of this substance is shown in Fig. 2, which indicates that it does not contain the tropone ring[12]. Since alkaline rearrangement of 2-phenyltropone results in the formation of diphenyl-2-carboxylic



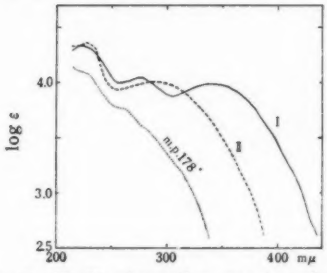


Fig. 2. U. V. absorption spectra of 2,7-diphenyl-(I), 2,3-diphenyltropone (II) and a compound melted at 178°C in methanol.

8) I. R. spectra are measured using Perkin-Elmer Model 21 double beam spectrophotometer by Mr. Yusaku Ikegami of the Chemical Research Institute Non-aqueous Solutions, to whom the author is deeply indebted.

9) T. Nozoe, T. Mukai and J. Minegishi, *Proc. Japan Acad.*, **27**, 419 (1951).

10) T. Mukai, to be published.

11) H. F. Bluhm, H. V. Donn and H. D. Zook, *J. Am. Chem. Soc.*, **77**, 4406 (1955).

12) M. Tsuboi, This Bulletin, **25**, 369 (1952).

13) T. Nozoe, T. Mukai and K. Takase, *Science Repts. Tohoku Univ.*, **I**, **39**, 164 (1956).

acid[1], the rearrangement product of II was assumed to be 2,3-diphenylbenzoic acid but mixed fusion with 2,3-diphenylbenzoic acid resulted in depression of the melting point. The reaction must have been quite specific and examination is still in progress as to the structure of this rearrangement product.

II easily forms a 2,4-dinitrophenylhydrazone and gives 2-amino-6,7-diphenyltropone (III) by reaction with hydrazine[1,13]. Alkali hydrolysis of III affords 3,4-diphenyltropolone (IV) which shows the coloration with ferric chloride characteristic of tropolone. The ultraviolet spectrum of IV, shown in Fig. 3, exhibits the

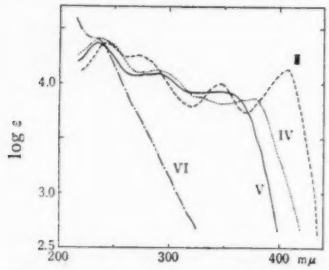
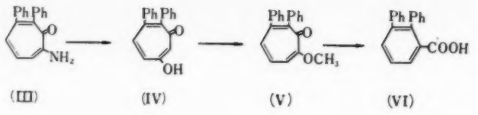


Fig. 3. U. V. absorption spectra of 2-amino-6,7-diphenyltropone (III), 3,4-diphenyltropolone (IV), methyl ether of IV (V) and 2,3-diphenylbenzoic acid (VI) in methanol.

maximum absorptions at 323 and 374 mμ, which is a tropolone type[14], but it seems to be different from that of 3-phenyltropolone[1] since there is a maximum at 285mμ. IV forms a methyl ether (probably V[15]) on the application of diazomethane and reaction of the methyl ether with sodium methoxide in absolute methanol, followed by hydrolysis gives compound VI of m. p. 162°C. 2,3-Diphenylbenzoic acid had already been synthesized by K. Alder et al.[16], who reported the compound to melt at 161°C, so that VI must be identical with it. From the series of reactions of II to VI, the structure of II was established as 2,3-diphenyltropone. Fig. 3 gives the ultraviolet spectra of V and VI.

Next, bromination of these two diphenyltropones was carried out. Application of



an excess of bromine to I in acetic acid first afforded a dibromide VII which, on treatment with sodium acetate, formed a monobromo compound VIII. Ultraviolet absorption spectra of VII and VIII are shown in Fig. 4. From the result of

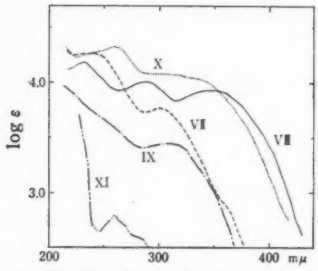


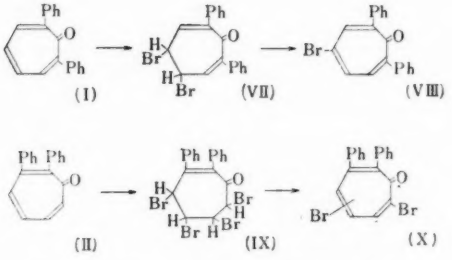
Fig. 4. U. V. absorption spectra of brominated compounds of I (VII and VIII), of II (IX and X) and 2,7-diphenylsuberone (XI) in methanol.

dehydrobromination of VII and the fact that the ultraviolet spectrum of VII is shifted to a shorter wave-length region, it is assumed that VII is a bromine addition product. On the other hand, the absorption curve of VIII is similar to that of I, shifted slightly to a longer wavelength region, which indicates that VIII may be considered to be a bromine substituted compound. Experiments to determine the position of the bromine were not carried out but it has been pointed out that bromine addition does not take place at the double bond with the phenyl group in the bromination of 2-phenyltropone[17]. It follows, therefore, that VII would be the compound with bromine addition in C_4—C_5 positions and VIII is most probably 4-bromo-2,7-diphenyltropone. In the ultraviolet spectrum of VII there is a certain amount of absorption in a region near 300 mμ, so that an unsaturated ketonic structure like VII seems to be most appropriate.

Bromination of II in acetic acid also results in the formation of a tetrabromide IX whose treatment with sodium acetate to effect dehydrobromination affords a dibromo compound X. Since there are two phenyl substituents in C_2 and C_3 position of II, it follows that bromine addition

14) T. Nozoe, *Fortshr. Chem. Org. Naturst.*, **13**, 232 (1956).
15) There are two isomers of methyl ether of 3-phenyltropolone, of which the ether corresponding to V was more easily isolated than another isomer. (cf. Ref. 1).
16) K. Alder, J. Haydn, K. Heimbach and K. Neufang, *Ann.*, **586**, 110 (1954).

must take place in C_4—C_5 and C_6—C_7 double bond[17]. Ultraviolet spectra of IX and X, indicated in Fig. 4, show that the absorption of IX is low in intensity and there is a good probability that it is an addition product, while the absorption of X is shifted to a longer wave-length region than that of II and it is probably safe to conclude that X is a bromine substituted compound.



Hydrogenation of I with palladium-charcoal as a catalyst resulted in facile absorption of hydrogen and 2,7-diphenylsuberone (XI) was formed. The ultraviolet spectrum of XI in Fig. 4 shows the absorption of two phenyl at groups 259mμ[18], while its infrared spectrum in Fig. 5 shows absorptions for C=O at 1706 for C=C of phenyl at 1603 and for CH_2 at 2935 and

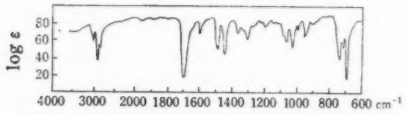


Fig. 5. I. R. absorption spectrum of 2,7-diphenylsuberone (XI) in KBr.

2860 cm^{-1}, which support the structure of XI. In connection with the examination of unsaturated characters, both I and II were heated with maleic anhydride in xylene but no addition products were obtained.

Experimental[19]

Reaction of 3-Phenyltropolone and Phenyllithium. — To a solution of phenyllithium, obtained from 0.42 g. (0.06 g. at.) of lithium, 4.7 g. (0.03 mole) of bromobenzene, and 18 cc. of ether, a solution of 1.00 g. (0.005 mole) of 3-phenyltropolone in 18 cc. of ether and 18 cc. of benzene was added dropwise in a nitrogen stream, when a slight generation of heat was observed. The mixture was stirred for 2 hours at room temperature and dilute sulfuric acid was then added under ice-cooling to effect decomposition. The organic solvent layer was separated, washed with water, and dried over anhydrous sodium sulfate. Evaporation of the solvent left 1.2 g. of dark reddish brown oil which was dissolved in benzene, saturated with hydrogen chloride gas, and the oily hydrochloride that separated out was collected by decantation. This was decomposed with water, extracted with carbon tetrachloride, and the solution was passed through an alumina column. Evaporation of the solvent from the initial carbon tetrachloride eluate afforded 180 mg. of colorless crystals I, m. p. 130~132°C. Recrystallization from ethanol gave colorless microplates, m. p. 133°C.

Anal. Found: C, 87.97; H, 5.65. Calcd. for $C_{19}H_{14}O$: C, 88.34; H, 5.46%. λ_{max}^{MeOH} mμ (log ε): 226(4.37), 279(4.06), 340(4.01).

The alumina column was then eluted with benzene and evaporation of benzene from the effluent left 130 mg. of colorless crystals II, m. p. 146°C. Recrystallization from ethanol afforded colorless plates, m. p. 147°C.

Anal. Found: C, 88.40; H, 5.47. Calcd. for $C_{19}H_{14}O$: C, 88.34; H, 5.46%. λ_{max}^{MeOH} mμ (log ε): 227(4.39), 292(4.03).

The benzene solution obtained after removal of the oily hydrochloride was washed with water and then passed through an alumina column. Initial effluent afforded 60 mg. of I, m. p. 132°C, and the latter eluate afforded further 110 mg. of II, m. p. 146°C. By mixed fusion, I was identified with the compound, m. p. 130°C, obtained from the reaction of 2-phenyltropone and phenylmagnesium bromide[4].

Addition of ethanolic solution of picric acid to the ethanolic solution of I or II failed to afford any crystalline picrate.

2, 4-Dinitrophenylhydrazone of II was obtained as dark red crystals, m. p. 208°C (decomp.).

Anal. Found: N, 12.24. Calcd. for $C_{25}H_{18}O_4N_4$: N, 12.87%. λ_{max}^{MeOH} mμ (log ε): 400(4.36).

On the other hand, application of 2, 4-dinitrophenylhydrazine to I in ethanol, in the presence of a trace of hydrochloric acid, with heating resulted in the recovery of I.

Behavior of the Diphenyltropones (I and II) to Alkali. — To a mixed solution of 1 cc. of ethanol and 1 cc. of 2N potassium hydroxide, 20 mg. of II was added and the mixture was refluxed on a water bath for 1.5 hours, when the solution turned brown in color and a small amount of resinous solid separated out. Ethanol was then evaporated from the mixture, water was added, and solid matter was filtered off. The filtrate was acidified and extracted with chloroform, from which 10 mg. of a pale yellow oil was obtained.

17) T. Mukai, *Science Repts. Tohoku Univ.*, **I**, **38**, 280 (1954).

18) In the series of Ph-$(CH_2)_n$-Ph (n>1), the absorption band is situated near 260 mμ and is not so intense (ε< 3000). cf. A. E. Gillam and E. S. Stern "An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry" Edward Arnold Ltd., London (1955), p. 64.

19) m.p. are uncorrected. The microanalyses were carried out by Mr. Shinichi Ohyama and Miss Ayako Iwanaga, to whom the author's deep gratitude is hereby expressed.

Trituration of this oil with cyclohexane effected crystallization and colorless crystals which sintered at 170°C and melted at 175~178°C were obtained. This substance showed depression of the melting point on admixture with 2, 3-diphenylbenzoic acid (VI), m. p. 162°C, described later.

On the other hand, the same treatment of 20 mg. of I with ethanolic potassium hydroxide solution by refluxing for 4 hours afforded 20 mg. of crystals melting at 124~126°C, indicating the complete recovery of I.

Reaction of the Diphenyltropones (I and II) with Hydrazine or Hydroxylamine. — *a*) To a solution of 40 mg. of II dissolved in 0.2 cc of ethanol, 0.10 cc. of 80% hydrazine hydrate was added and the mixture was heated on a water bath for a few minutes, from which 30 mg. of 2-amino compound was obtained as yellow crystals of m. p. 253~258°C. Recrystallization from ethanol afforded gold colored scales III, m. p. 260°C.

Anal. Found: C, 83.36; H, 5.67; N, 5.92. Calcd. for $C_{19}H_{15}ON$: C, 83.49; H, 5.53; N, 5.13%. λ_{max}^{MeOH} mμ (log ε): 242(4.35), 270(4.25), 346(4.00), 406(4.14).

I is recovered on reacting with hydrazine in the same manner. Application of hydrazine to a mixture of I and II results in amination of II alone and I remains inactive. This is a convenient method for the separation of I and II.

b) A mixture of 30 mg. of II, 30 mg. of hydroxylamine hydrochloride, 0.5 cc. of absolute ethanol, and 0.5 cc. of pyridine was refluxed on a water bath for 2 hours. The solvent was evaporated from the mixture and water was added, from which 25 mg. of yellow crystals, m.p. 210~213°C, separated out. Recrystallization from ethanol raised the melting point to 212~213°C.

Anal. Found: N, 4.80. Calcd. for $C_{19}H_{15}ON$: N, 5.13%. λ_{max}^{MeOH} mμ (log ε): 237(4.30), 345(3.70), 403(3.72).

From the color of this substance and its ultraviolet spectrum, it is still doubtful that the substance is an oxime and probable occulusion of III may be considered.

I is completely recovered on similar reaction with hydroxylamine.

Formation of 3, 4-Diphenyltropolone (IV). —A solution of 120 mg. of III dissolved in a mixture of 6 cc. of ethanol and 2 cc. of 6N potassium hydroxide was refluxed for 12 hours on a water bath, ethanol was evaporated, and the residue was diluted with water. The solution was neutralized to weak acidity, extracted with benzene, and evaporation of benzene left 110 mg. of pale brown crystals, m. p. 160~165°C. Recrystallization from ethanol afforded 100 mg. of colorless crystals (IV), m. p. 173°C.

Anal. Found: C, 83.08; H, 5.41. Calcd. for $C_{19}H_{14}O_2$: C, 83.20; H, 5.15%. λ_{max}^{MeOH} mμ (log ε): 235(4.39), 285(4.11), 323(3.87), 365(3.83), 374(3.85).

Formation of 3, 4-Diphenyltropolone Methyl Ether (V) and Aromatization Rearrangement of V.—To 2cc. of 2.8% ethereal solution of diazomethane, 70 mg. of IV was added and the mixture was allowed to stand in a cool place overnight. Evaporation of ether left 70 mg. of pale yellow needles, m. p. 140~144°C. Recrystallization from benzene-cyclohexane mixture gave V melting at 147°C.

Anal. Found: C, 83.31; H, 5.39. Calcd. for $C_{20}H_{16}O$: C, 83.31; H, 5.59%. λ_{max}^{MeOH} mμ (log ε): 235(4.37), 287(4.09), 355(3.89).

A solution of 60 mg. of the methyl ether V dissolved in 3 cc. of absolute ethanol containing 10 mg. of sodium was refluxed for 30 hours, 5 cc. of water was added, and the mixture was heated again for 3 hours. Ethanol was evaporated, the residue diluted with water, and the solution extracted with ether to remove any neutral substance. The aqueous layer was neutralized until the acidity was weak, extracted with benzene, and benzene residue gave 20 mg. of colorless crystals. Recrystallization from benzene-cyclohexane mixture afforded colorless crystals, m. p. 162°C. Reported m. p. for 2, 3-diphenylbenzoic acid is 161°C[16].

Bromination of 2, 7-Diphenyltropone (I).— A mixture of 200mg. of I and 300mg. (5mol. equiv.) of bromine in 7 cc. of acetic acid was warmed, sealed in a vessel, and allowed to stand for 6 days. Evaporation of acetic acid over potassium hydroxide in a vacuum desiccator left 100 mg. of pale yellow crystals, m. p. 115°C (decomp.). The filtrate obtained after removal of these crystals was evaporated and 150 mg. of orange solid obtained.

Its treatment with ethanol and benzene afforded 60mg. of pale yellow crystals, m.p. 123°C (decomp.).

Recrystallization of the former from ethanol and benzene gave 80 mg. of pale yellow needles VII, m. p. 120°C (decomp.), which were identified with the later-obtained crystals by mixed fusion.

Anal. Found: C, 54.58; H, 3.41. Calcd. for $C_{19}H_{14}OBr_2$: C, 54.78; H, 3.34%. λ_{max}^{MeOH} mμ (log ε): 227(4.28).

Dehydrobromination of the Bromine Adduct VII.—A mixture of 60 mg. of VII and 50 mg. of sodium acetate in 2 cc. of ethanol and 1 cc. of benzene was refluxed for 2 hours, the solvent was evaporated, and the residue was diluted with water. Extraction with benzene and evaporation of solvent afforded 50 mg. of orange yellow oil which solidified to crystals of m. p. 75~80°C. The carbon tetrachloride solution of this substance was passed through an alumina column and the crystals obtained therefrom were recrystallized from ethanol to pale yellow needles VIII, m. p. 92°C.

Anal. Found: C, 68.01; H, 3.91. Calcd. for $C_{19}H_{13}OBr$: C, 67.60; H, 3.89%. λ_{max}^{MeOH} mμ (log ε): 229(4.20), 285(4.01), 347(3.94).

Bromination of 2, 3-Diphenyltropone (II). —To a solution of 50 mg. of II dissolved in 3 cc. of acetic acid, 130 mg. (4.5 mol. equiv.) of bromine was added and the mixture was allowed to stand for 3 days at room temperature. Acetic acid was evaporated over potassium hydroxide and 100 mg. of orange crystals, m. p. 123~130°C, was obtained. Recrystallization from benzene-cyclohexane mixture afforded pale yellow plates IX, m. p. 131°C (decomp.).

Anal. Found: C, 40.35; H, 2.74. Calcd. for $C_{19}H_{14}OBr_4$: C, 39.41; H, 2.41%. λ_{max}^{MeOH} mμ (log ε): 305(3.43).

Dehydrobromination of Bromine Adduct IX.—To a solution of 60 mg. of IX dissolved in 2 cc. of ethanol, 30 mg. of sodium acetate was added and the mixture was refluxed on a water bath for 3 hours. Ethanol was evaporated from the mixture, the residue was dissolved in carbon tetrachloride, and the solution was passed through an alumina column. The column was eluted with benzene, and evaporation of the solvent from both eluates left 30 mg. of pale yellow crystals, m. p. 135~160°C, which recrystallized from ethanol to pale yellow needles X, m. p. 167°C.

Anal. Found: C, 55.19; H, 3.01. Calcd. for $C_{19}H_{12}OBr_2$: C, 54.81; H, 2.88%. λ_{max}^{MeOH} mμ (log ε): 258(4.33), 295(4.10).

Catalytic Reduction of 2,7-Diphenyltropone (I).—To a solution of 50 mg. of I dissolved in 10 cc. of methanol, 10 mg. of 5% palladium-charcoal was added and the mixture was hydrogenated at ordinary temperature and pressure. The absorption of hydrogen ceased after 14.2 cc. (3 mol. equiv.) of hydrogen had been absorbed. The filtrate obtained after removal of the catalyst was evaporated and the colorless crystalline residue was recrystallized from methanol to 40 mg. of colorless needles XI, m. p. 143°C.

Anal. Found: C, 86.72; H, 7.11. Calcd. for $C_{19}H_{20}O$: C, 86.32; H, 7.63%. λ_{max}^{MeOH} mμ (log ε): 259(2.79).

Diels-Alder Reaction of the Diphenyltropones (I and II).—A solution of 50 mg. of I and 40 mg. of maleic anhydride dissolved in absolute xylene was refluxed for 4 hours and the residue obtained on evaporation of the solvent was recrystallized from ethanol to 40 mg. of pale yellow crystals, m. p. 132°C, identified with I by mixed fusion.

The same treatment of II with maleic anhydride also resulted in its recovery but the amount recovered was smaller. However, no addition product was obtained even in this case.

The author takes this opportunity to express his sincere gratitude to Professor Tetsuo Nozoe for his kind guidance throughout the course of this work, to Professor Shuichi Seto for much helpful advice and to Mr. Mutsuo Ishii for his cooperation in carrying out the experiments.

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Synthesis of α-Substituted Glutarimides via Michael Condensation

By Tetsuji KAMETANI[1], William TAUB and David GINSBURG

(Received May 10, 1958)

A useful approach to the synthesis of certain α-substituted glutarimides required as intermediates in the synthesis of potential hypnotic compounds appeared to be the Michael condensation of appropriate donors to various nitrogen containing acceptors.

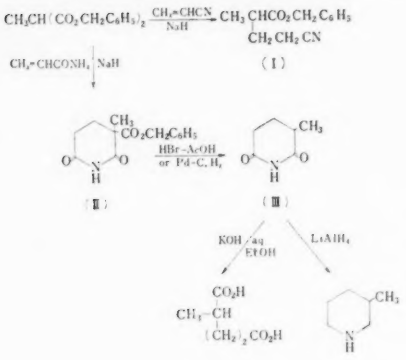
Cyanoethylation of dibenzyl methyl malonate in the presence of sodium hydride gave the appropriate Michael condensation adduct I whose conversion into α-methylglutarimide (III) proved inefficient. However, it was found that when acrylamide was used as acceptor, a glutarimide derivative II was isolated directly upon workup of the Michael reaction mixture. The α-methyl-α-carbobenzoxy-glutarimide (II) thus obtained could be debenzylated either by catalytic hydrogenolysis using palladized carbon or by treatment with hydrogen bromide in acetic acid[2], affording α-methyl-glutarimide (III). The structure of the latter was proven by hydrolysis to yield the known α-methylglutaric acid and by lithium aluminium hydride reduction to yield the known 3-methylpiperidine. These reactions are summarized in reaction scheme 1.

Similarly, cyanoethylation of diphenylacetonitrile in the presence of methanolic potassium hydroxide gave α,α-diphenylglutarodinitrile (IV). The transformations of this product into α,α-diphenylglutarimide (V) and intermediate products are summarized in reaction scheme 2.

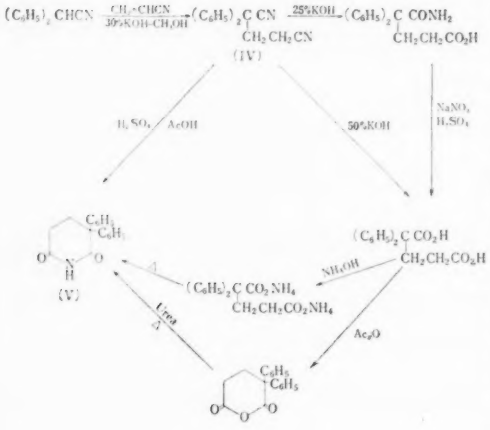
1) Present Address, Pharm. Faculty, the University of Osaka, Hotarugaike, Toyonaka, Osaka.

2) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

Reaction Scheme 1.



Reaction Scheme 2.



Experimental

Dibenzyl methylmalonate.—This was prepared analogously to the preparation of dibenzyl malonate[3] by transesterification of diethyl methylmalonate[4] (58 g.; 0.33 mole) with benzyl alcohol (72 g.; 0.67 mole) in dry benzene (660 ml.) in the presence of sodium methoxide (1.7 g.). The ester had b. p. 164～167°C/0.04 mm.Hg (71 g.; 72%).

Benzyl α-(2′-cyanoethyl)-propionate (I).—The sodio-derivative was prepared by heating under reflux dibenzyl methylmalonate (37 g.) and sodium hydride (3.8 g.) in dry benzene (300 ml.) for 6 hr. The reaction mixture was cooled and a solution of acrylonitrile (8 g.) in dry benzene (50 ml.) was added and the whole was again refluxed for 6 hr. The benzene was removed at the water pump and the residue was poured into water containing sufficient acetic acid for neutralization. The oily precipitate was extracted with ethyl acetate, the extract was dried, and the solvent was evaporated. Ether was added to the residue (46 g.) and the extract was filtered to remove yellow polymeric material. The ether-soluble material (33 g.) solidified after long standing and was recrystallized from benzene-petroleum ether, m. p. 79～80°C.

Anal. Found: C, 76.85; H, 6.37; N, 4.43. Calcd. for $C_{13}H_{15}O_2N \cdot C_6H_6$: C, 77.28; H, 7.11; N, 4.74%.

3-Carbobenzoxy-3-methylglutarimide (II).—A mixture of dibenzyl methylmalonate (4 g.), sodium hydride (0.33 g.) and dry benzene was refluxed for 2 hr. After cooling, acrylamide[5] (0.33 g.) was added and the mixture was refluxed for 4～5 hr. A clear solution was finally obtained. The benzene was removed under reduced pressure and the residue was poured into ice water containing the calculated quantity of acetic acid required for neutralization. Scratching the precipitated oil after the addition of a small amount of ethanol induced crystallization. The crude solid (3 g.) was filtered, m. p. 93～94°C (from aqueous ethanol).

Anal. Found: C, 64.53; H, 5.61; N, 5.10. Calcd. for $C_{14}H_{15}O_4N$: C, 64.36; H, 5.79; N, 4.36%.

3-Methylglutarimide (III).—*a*) The imide-ester II (36.9 g.) was refluxed with 30% hydrogen bromide in acetic acid (200 ml.) for 6 hr. After evaporation to dryness, the residue was treated with aqueous sodium bicarbonate and ether. The ether layer was evaporated in vacuo to remove the solvent and benzyl bromide. A small amount of imide (0.6 g.) crystallized in the residue. The bicarbonate solution was acidified with dilute hydrochloric acid. The imide was extracted with ether and afforded more crystalline material on evaporation (4.1 g.). Continuous extraction of the acidified solution with ether afforded more of the imide (6.7 g.). Total yield, 62%, m. p. 101～102°C (from ethanol-ether). The imide can be sublimed in a vacuum.

Anal. Found: C, 56.40; H, 7.24; N, 11.10. Calcd. for $C_6H_9O_2N$: C, 56.68; H, 7.14; N, 11.02%.

b) The imide can also be obtained by hydrogenolysis of the imide-ester II (5 g.) in ethyl acetate (100 ml.) in the presence of 10% palladized carbon (0.5 g.) at an initial pressure of 48 lbs./sq. in metric system. Hydrogen uptake (1 mole) was complete within 30 min. The catalyst was removed by filtration and the solvent was removed by distillation. The residue soon solidified, m. p. 94～95°C. Recrystallization from ethanol-ether gave the imide III, m. p. 101～102°C, identical with that described above.

Proof of Structure of 3-Methylglutarimide.—*a*) The imide III (100 mg.) was saponified by refluxing for 7 hr. with potassium hydroxide (0.52 g.) in 50% ethanol (4.5 ml.). Ammonia was evolved. The mixture was evaporated to dryness and the residue was taken up in a little water and the solution was acidified and extracted with ether. The ether was removed from the

3) D. Ginsburg and R. Pappo, *J. Am. Chem. Soc.*, **75**, 1094 (1953).

4) "Organic Syntheses", Coll. Vol. II, Wiley, New York, (1946), p. 279.

5) Cf. D. Elad and D. Ginsburg, *J. Chem. Soc.*, **1953**, 4137.

extract and the residual oil which soon solidified (100 mg.) was recrystallized from benzene-petroleum ether, m. p. 75°C. Lit.[6] m. p. 77~80°C for α-methylglutaric acid.

b) A solution of the imide III (1.27 g.) in dry dioxan (20 ml.) was added to a solution of lithium aluminium hydride (0.27 g.) in dry ether (30 ml.) during 20 min. The mixture was refluxed for 90 min. and was cooled and decomposed slowly with hydrochloric acid (10%). The mixture was evaporated to dryness under reduced pressure. Aqueous potassium hydroxide (50%) was added and the mixture was extracted with ether. Removal of the ether gave an oil (0.3 g.) of piperidine-like odor. The hydrochloride was prepared and had m. p. 171~172°C (from ether-benzene). Lit.[7] m. p. 171~172°C for 3-methylpiperidine.

α,α-Diphenylglutaronitrile (IV).—To a solution of diphenylacetonitrile (77.5 g.) in *tert*-butanol (120 ml.) and dioxan (30 ml.) was added methanolic potassium hydroxide (30%; 3 ml.). A solution of acrylonitrile (21.2 g.) in *tert*-butanol (200 ml.) was then added with stirring at 10~25°C during 30 min. Stirring at room temperature was continued for 2 hr. The emulsion deposited much crystalline material. The mixture was poured into ice-water and the solid was filtered and recrystallized from dilute methanol, m. p. 69~70°C (87 g.). The product depressed the m. p. of diphenylacetonitrile.

Anal. Found: C, 82.82; H, 5.72; N, 11.06. Calcd. for $C_{17}H_{14}N_2$: C, 82.90; H, 5.73; N, 11.37%.

γ-Carboxamido-γ,γ-diphenylbutyric acid.—This was obtained by refluxing the dinitrile (2.46 g.) with potassium hydroxide (2.46 g.) in aqueous (10 ml.) ethanol (10 ml.) for 17 hr. followed by the addition of more potassium hydroxide (5 g.) and continued reflux for 23 hr. longer. The ethanol was evaporated, dilute hydrochloric acid was added and the precipitate was recrystallized first from methanol, then from benzene, m. p. 153~154°C (1.19 g.).

Anal. Found: C, 72.13; H, 5.97; N, 5.04. Calcd. for $C_{17}H_{17}O_3N$: C, 72.06; H, 6.05; N, 4.94%.

α,α-Diphenylglutaric acid.—*a*) The dinitrile (2.46 g.) was saponified by refluxing with aqueous potassium hydroxide (50%; 5.6 g.) for 4 hr. After dilution with water and hydrochloric acid the resinous solid which separated was recrystallized from aqueous methanol, m. p. 187~188°C (1.77 g.).

Anal. Found: C, 72.35; H, 6.15. Calcd. for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67%.

b) The above amido-acid (0.14 g.) was dissolved in concentrated sulfuric acid (0.6 g.) and a saturated solution of sodium nitrite (0.4 g.) was added beneath the surface of the yellow acid solution. The mixture was warmed on the steam bath for 1 hr. It was poured into ice water and the precipitate was filtered. Recrystallization from aqueous methanol gave α,α-diphenylglutaric acid (30 mg.) identical with that described above.

α,α-Diphenylglutaric anhydride.—A solution of the above diacid (4.26 g.) and acetic anhydride (25 ml.) was refluxed for 90 min. The solvents were removed under reduced pressure and the residue was recrystallized from benzene-petroleum ether (3 g.; 2 crops), m. p. 134°C.

Anal. Found: C, 76.99; H, 5.38. Calcd. for $C_{17}H_{14}O_3$: C, 76.67; H, 5.30%.

α,α-Diphenylglutarimide.—*a*) A mixture of the dinitrile IV (24.6 g.), concentrated sulfuric acid (50 g.), acetic acid (35 g.) and water (15 ml.) was refluxed for 3 hr. It was poured into ice-water and the viscous solid was triturated with a little methanol and then washed with a little water and filtered (18.2 g.), m. p. 169~170°C (sinters 160°C) (from methanol). The imide is soluble in potassium hydroxide solution, but not in sodium bicarbonate solution.

Anal. Found: C, 77.02; H, 5.76; N, 5.39. Calcd. for $C_{17}H_{15}O_2N$: C, 76.96; H, 5.70; N, 5.28%.

b) A mixture of the above anhydride (1.33 g.) and urea (3 g.) formed a clear melt when heated to 140~150°C in an oil bath. The temperature was raised to 200~210°C and the contents solidified after 1 hr. at this temperature. After cooling and extraction with water to remove excess urea, the undissolved residue was recrystallized from aqueous methanol, m. p. 169°C (sinters 161°C) (0.76 g.). This product was identical with the imide described above.

c) α,α-Diphenylglutaric acid was dissolved in excess ammonium hydroxide and the viscous ammonium salt (1.67 g.) was obtained by evaporation to dryness. This was heated in a distillation flask with a free flame and some material distilled at 190°C. Ether was added to the distillate and petroleum ether was then added, inducing crystallization of the imide (0.1 g.) identical with that described above, m. p. 168~169°C.

6) A. Franke and M. Kohn, *Monatsh.*, **23**, 740 (1920).
7) A. Franke and M. Kohn, ibid., **23**, 884 (1902).

Summary

A method for the synthesis of α-monosubstituted and α,α-disubstituted glutarimides by Michael addition of active methylene and methine compounds to acrylonitrile or acrylamide, is described.

One of the authors (T. K.) thanks Calanda Foundation for the award of Fellowship.

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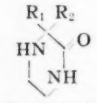
Synthesis of 3,3-Disubstituted-2-Piperazinones

By Tetsuji KAMETANI[1], William TAUB and David GINSBURG

(Received May 10, 1958)

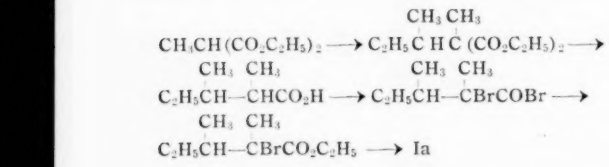
It was believed that 2-piperazinones containing a quaternary carbon atom adjacent to the carbonyl group might exhibit hypnotic activity. Several 3,3-disubstituted-2-piperazinones were therefore synthesized essentially by the method of Aspinall[2] although with some modification (see experimental section). Although the desired activity was present in several of the compounds, it did not appear sufficient for further extention.

The 3-methyl-3-*sec*-butyl compound Ia was prepared



(I) a. R=Me; R_2=*sec*-Bu
b. $R_1=R_2$=Ph
c. R_1=Ph; R_2=Et

in accordance with the reaction scheme.



The 3,3-diphenyl derivative Ib was prepared by treatment of methyl α-bromodiphenylacetate resulting from treatment of methyl diphenylacetate with *N*-bromosuccinimide, with ethylene diamine.

The 3-phenyl-3-ethyl derivative (Ic) was prepared by bromination of methyl α-ethyl phenylacetate with *N*-bromo-succinimide followed by treatment of the resulting bromo-ester with ethylene diamine.

Experimental

Diethyl methyl-*sec*-butylmalonate was prepared in 73% yield by alkylation of diethyl methylmalonate[3] with 2-bromobutane, in the presence of sodium ethoxide. The ester boils at 110~123°C/19 mmHg.

α-*sec*-Butylpropionic acid was prepared in 66% yield by refluxing the above ester with 20% aqueous ethanolic potassium hydroxide for 3 hrs., acidification with 10% hydrochloric acid, ether extraction and decarboxylation of the crude malonic acid at 200°C.

α-Bromo-α-*sec*-butylpropionyl bromide was obtained in 86% yield[4] by treating the above acid (6.8 g.) in the usual way with red phosphorus (0.68 g.) and bromine (6.8 ml.), b. p. 114~121°C/40 mmHg.

Ethyl α-bromo-α-*sec*-butylpropionate was prepared in 68% yield by the usual procedure from the freshly distilled acid bromide (25.5 g.) and dry ethanol (4.4 g.), b. p. 116°C/33 mmHg.

3-Methyl-3-*sec*-butyl-2-piperazinone (Ia).—A solution of ethyl α-bromo-α-*sec*-butylpropionate (15.4 g.) in dry ethanol (100 ml.) was added dropwith with stirring at roon temperature during 3 hr. to a solution of ethylene diamine (50 g.) in dry ethanol (150 ml.). After 2 hr. longer at reflux temperature, a solution of sodium ethoxide (1.51 g.) of sodium in dry ethanol (30 ml.) was added to the boiling solution during 30 min. Refluxing was continued for two additional hours. Excess ethanol and ethylene diamine were removed at the water pump. Acetone was added to the residue and the colorless precipitate was filtered. The mother liquor was evaporated and acetone was again added and the residue was distilled in 0.2 mm. It crystallized on standing, m.p. 58~61°C (from petroleum ether).

Anal. Found: C, 63.35; H, 10.62; N, 17.13. Calcd. for $C_{19}H_{18}ON_2$: C, 63.49; H, 10.66; N, 16.46%. The hydrobromide had m.p. 203~206°C (from absolute ethanol-ether).

When an ether solution of the α-bromo-acid bromide was added to a solution of excess ethylene diamine in chloroform at 0°C, a colorless precipitate was formed. After refluxing for 3 hrs. a brown insoluble oil was obtained and this solidified on standing. Recrystallization from benzene gave a product, m.p. 172~174°C which was insoluble in water and gave a negative test with aqueous silver nitrate. Its analysis was unsatisfactory but there is indication that this product is the α,α-dibromo-bis-amide, $[CH_3C(Br)(sec\text{-}C_4H_9)\text{-}CONHCH_2]_2$.

Anal. Found: C, 44.30; H, 6.84; N, 7.13; Br, 34.73. Calcd. for $C_{16}H_{30}O_2N_2Br_2$: C, 43.44; H, 6.78; N, 6.35; Br, 36.19%.

Methyl diphenylacetate was prepared from

1) Present Adress, Pharm. Faculty, the University of Osaka, Hotarugaike, Toyonaka, Osaka.

2) S. R. Aspinall, *J. Am. Chem. Soc.*, **62**, 1202 (1940). *cf.* R. L. Hodgson, I. R. C. Bick and D. J. Cram, ibid., **76**, 1138 (1954).

3) "Organic Syntheses", Coll. Vol. II, Wiley, New York, (1946), p. 279.

4) Ibid., Vol. 33, Wiley, New York, (1953), p. 29.

diphenylacetic acid (25 g.), methanol (500 ml.) and concentrated sulfuric acid (25 ml.). The mixture was refluxed for 3 hrs., most of the methanol was removed at the water pump and the residue was poured into ice-water. The oil which soon solidified was recrystallized from aqueous ethanol, m.p. 60°C (24.8 g.). Lit.[5] m.p. 60.°C.

Methyl α-bromodiphenylacetate.—A mixture of the above methyl ester (2.26 g.), carbon tetrachloride (15 ml.) and *N*-bromosuccinimide (1.82 g.) was refluxed for 6 hrs. After the usual workup the bromo-ester was obtained (2.77 g.; 91%) as an oil which was used in its crude form.

3,3-Diphenylpiperazinone (Ib).—A mixture of the crude bromoester (2.5 g.), ethylene diamine (1.2 g.) and dry chloroform (10 ml.) was refluxed for 4 hrs. After standing overnight the red oil which had separated solidified and appeared to be the hydrobromide of ethylene diamine. The chloroform solution was evaporated to dryness and the glassy residue was triturated with benzene, affording colorless crystals (2.3 g.; 96%), m.p. 163°C (from dry benzene; sinters 155~156°). The product gives a picrate, m.p. 248~249°C (from ethanol).

Anal. Found: C, 76.20; H, 6.488; N, 11.18. Calcd. for $C_{16}H_{16}ON_2$: C, 76.16; H, 6.39; N, 11.10%.

Methyl α-phenylethylate was obtained in 91% yield by esterification of the acid as described above for methyl diphenylacetate. It had b.p. 225~228°C. Lit.[6] b.p. 225~226°C.

Methyl α-bromo-α-phenylethylacetate was obtained by bromination of the above ester (17.8 g.) with *N*-bromosuccinmide (20.8 g.) in carbon tetrachloride (100 ml.) during 4 hr. After the usual workup the crude ester (94% yield) was used in order to avoid dehydrobromination during distillation at a relatively high temperature.

3-Ethyl-3-phenylpiperazinone (Ic). — This was prepared in poor yield from the crude bromo-ester and ethylene diamine as described for the preparation of Ia. It was an oil, b.p. 140~160°C/0.4 mmHg. (1g. from 9.7g. bromo-ester).

Anal. Found: N, 14.01. Calcd. for $C_{12}H_{16}ON_2$: N, 13.72%.

Summary

Several 3, 3-disubstituted-2-piperazinones have been synthesized for pharmacological testing as potential hypnotics.

One of the authors (T. K.) thanks Calanda Foundation for the award of Fellowship.

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5) P. Fritsch and F. Feldmann, *Ann.*, **306**, 81 (1899).

6) M. M. Rising and T. W. Zee, *J. Am. Chem. Soc.*, **50**, 1211 (1928).

Infrared Studies of Formic Acid, Chemisorbed on Copper, Nickel and Zinc

By Kozo Hirota, Keiji Kuwata and Yasuo Nakai

(Received March 25, 1958)

The catalytic decomposition of formic acid into hydrogen and carbon dioxide, $HCOOH \rightarrow H_2 + CO_2$, has been studied kinetically by many researchers, and the mechanisms of the decomposition were often suggested in recent years[1]. However, it may be admitted that these mechanisms are established on the kinetic data without any exact knowledge of the adsorbed state of formic acid. Considering such situations, the present authors attempted to solve the problem by the infrared absorption technique which had been explored by Eischens et al.[2], i. e., the infrared spectra of formic acid adsorbed on metal surface were measured, and were compared with those of gaseous formic acid and solid formates.

Experimental

The catalysts studied were copper, nickel and zinc, each being dispersed in the extra fine powder of silica. Each metal catalyst was prepared by the thermal decomposition of the corresponding formate, followed by its reduction

1) a) G. M. Schwab and E. Schwab-Agallidis, *J. Am. Chem. Soc.*, **71**, 1806 (1949). b) D. A. Dowden et al., *Trans. Faraday Soc.*, **42**, 680 (1948). c) G. Rienäcker and N. Hansen, *Z. Elektrochem.*, **60**, 887 (1956).

2) R. P. Eischens, S. A. Francis and W. A. Pliskin, *J. Phys. Chem.*, **60**, 194 (1956).

with hydrogen at 200°C. The spectrum was measured at room temperature with Hilger H-800 spectrometer. The cell, which was constructed so as to be heated from the outside to reduce the catalyst enclosed, was laid horizontally before the slit of the spectrometer. The cell was made of pyrex glass and was similar to that described by Eischens. The windows of the cell and the thin plate supporting the catalysts were made of sodium chloride. It was ascertained previously that all the metal-silica-catalysts had no absorption bands in the spectral region concerned, and also that there was no absorption band caused by the interaction of formic acid with the windows and plates of sodium chloride in the same region.

Results

The absorption spectra of chemisorbed formic acid could be obtained from the bands which remained when the excess of free formic acid was desorbed by evacuating the cell until the strongest absorption band of free formic acid at 1740 cm^{-1} disappeared. These bands of chemisorbed formic acid, as shown in Table I, do not coincide with those of gaseous formic acid (1740 cm^{-1} and 1380 cm^{-1}). Typical spectra of formic acid chemisorbed on copper and zinc are shown in Fig. 1 and Fig. 2, where the pressure of gaseous formic acid is reduced successively in the order a, b and c. It is found generally that the absorption intensity of these bands increases in comparison with those of gaseous formic acid, according as its pressure decreases.

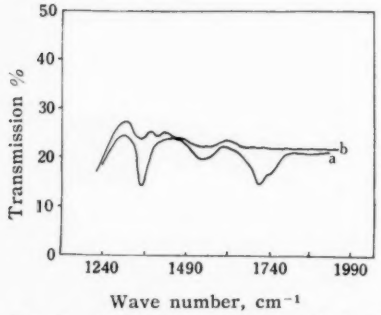

Fig. 1. Absorption spectrum of formic acid chemisorbed on copper catalyst.

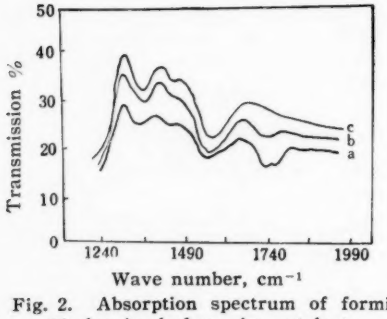

Fig. 2. Absorption spectrum of formic acid chemisorbed on zinc catalyst.

TABLE I

INFRARED ABSORPTION BANDS OF FORMIC ACID CHEMISORBED ON COPPER, NICKEL AND ZINC, OF THE CORRESPONDING SOLID METAL FORMATES AND OF FORMATE ION (cm^{-1})

Modes of vibration	Formic acid chemisorbed on			Metal formates of			Formate ion[3]
	Cu	Ni	Zn	Cu	Ni	Zn	
ν_4	1560	1600	1590	1580	1570	1580	1585
ν_5	1400	1380	1360(?)	1400	1400	1400	1385
ν_2	1360	(?)		.....[a]	.....[a]	.....[a]	1351

a) As Nujol oil was used, these bands were masked.

In the table, the bands of metal formates $(HCOO)_2Cu \cdot 2H_2O$, $(HCOO)_2Ni \cdot 2H_2O$ and $(HCOO_2)Zn \cdot xH_2O$ obtained are shown for the sake of reference. The spectra of these formates coincide fairly well with those of several formates in the literature[4]. Since the accuracy in measuring wave number of the maximum absorption was low, predominantly owing to weakness of the absorption, the last figures in the table were round figures.

Discussion

In each catalyst, two or three bands were observed. In Table I they are given the notations of the ν_4, ν_5 and ν_2 vibrations, because they seem to correspond to the antisymmetric ν_4 and ν_5 vibrations and to the symmetric ν_2 vibration of formate ion*, respectively. However, the other two symmetric vibrations, designated as ν_1 and ν_3, which will appear near 2825 cm^{-1} (ν_1) and 775 cm^{-1} (ν_3), were not observed. The ν_1 vibration would be masked by the strong and broad absorption bands of silica, while the ν_3 vibration would be hard to detect because of the small transmission of the sample in this region, even if there is. Moreover, it is noteworthy

3) Landolt-Börnstein, "Zahlenwerte und Funkionen", 1, 2 Teil, p. 237 (1951).

* Theoretical assignment of the vibrations of formate ion was given by T. Miyazawa, *J. Chem. Soc. Japan, Pure Chem. Sec.*, (*Nippon Kagaku Zassi*), **77**, 381 (1956).

4) Ll. Puval, H. Gerding et J. Lecomte, *Rec. Trav. Chim.*, **69**, 391 (1950).

that the ν_4, ν_5 and ν_2 vibrations are similar to those of the corresponding metal formates as shown in the table and also to those observed in the liquid solution[4]. Therefore the new bands caused by the adsorbed formic acid may be ascribed to the formate ion present on the surface of the catalysts.

According to the X-ray analysis of these crystals[5] and the spectrochemical studies of their microcrystals[6], a carbon atom combines with a metal atom via an oxygen atom in those crystals which are composed of formate groups (HCOO), metal atoms and water molecules. If the above structure can be extended to such surface complexes, it will be reasonable to assume that the formic acid is not physically adsorbed, but is chemisorbed, dissociating into the hydrogen atom and the formate group. Considering the structure of the metal formates[5], it is possible that the formate ion forms a bridged bond with two metal atoms on the surface. However, the ionic degree of the bond between the oxygen atom and the catalyst cannot be estimated in the present state of experimental accuracy.

It must be pointed out that the ν_5 vibration of formic acid chemisorbed on zinc is a little different from that of formate ion (1385 cm^{-1}), the difference being 75 cm^{-1}. It was supposed that it might owe to the imperfect reduction of the catalyst. However, as the difference decreased to 55 cm^{-1} when an amount of oxygen was chemisorbed, the deviation may be ascribed to some causes other than the chemisorption of oxygen, e. g., the mutual interaction between the chemisorbed chemical species, as pointed out by Eischens[2] and Yang[7]. Concerning this point, further research is required.

The above conclusion supports the "dissociation mechanism" of the Rienäcker[1c], i. e.,

$$HCOOH + H^+ + 2e \rightarrow H_2 + HCOO^- \quad (1a)$$

$$HCOO^- + [Met] \rightarrow CO_2 + [Met]^- + H \quad (1b)$$

but rejects the "monomolecular-mechanism", the essential feature of which is depicted in Fig. 3: i. e.,

$$HCOOH \rightarrow H_2 + CO_2 \quad (2)$$

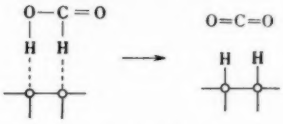

Fig. 3. Eley's Mechanism.

This latter mechanism, which was first proposed for the oxide catalysts by Schwab et al.[1a], was also proposed by Eley et al.[8] in the case of gold-palladium catalysts. Besides the above-mentioned reason, there is another one against Eley's mechanism from the standpoint of the structure of formic acid molecule[3,9], because the chemisorbed formic acid molecule would require higher potential energy to keep such a distorted structure.

It is interesting to mention that the kinetic studies[10] on the decomposition of formic acid on silver catalyst performed by one of the present authors (K. H.) also support the dissociation mechanism; i. e. in the intermediate state of the decomposition at the pressure of 2~3 cm Hg, more hydrogen was always found to exist than carbon dioxide at the temperature between 80~130°C, the maximum deviation of the ratio H_2/CO_2 being ca. 30%. If the decomposition had proceeded monomolecularly in one step as shown in Eq. 2, such a result would surely not have been found, because the decomposition was carried out on the condition that the adsorption of carbon dioxide was negligibly small and the addition of carbon dioxide in the original gas had no effect on the progress of the decomposition.

Therefore, for the several reasons cited above it seems that the dissociation mechanism is better than that of Eley, at least in the case of the metal catalysts. However, Schwab's mechanism may be justified in the case of the oxide catalysts which can act partly as dehydrogenation catalysts and partly as dehydration catalysts. Of course, it can not be asserted from the present experimental data that the free formate ion, $HCOO^-$, as described in Rienäcker's paper, exists on the metal surface.

5) R. Kiriyama, H. Ibamoto and K. Matsuo, *Acta Cryst.*, **2**, 482 (1954).
6) S. Yamada, H. Nakamura and R. Tsuchida, This Bulletin, **30**, 953 (1957).
7) A. C. Yang and C. W. Garland, *J. Phys. Chem.*, **61**, 1504 (1957).
8) D. D. Eley and P. Lucky, *Trans. Faraday Soc.*, **51**, 1483 (1957).
9) S. H. Bauer and R. M. Badger, *J. Chem. Phys.*, **5**, 852 (1937).
10) K. Hirota and T. Otaki, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), to be published (Accepted March 25,1958).

Conclusion

By the use of the infrared absorption technique, it can be concluded that formic acid, chemisorbed on copper, nickel and zinc, dissociates into two groups as HCOO and H at room temperature. Such a conclusion conforms with Rienäcker's mechanism on the dehydrogenation of formic acid. However, it can not be determined at the present state of the study whether or not these groups are linked to the metal atoms by ionic or covalent bonds.

The detailed discussion will be published in near future in addition to the data of kinetic research.

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Studies on the Interaction of Surface Films with Solute in Solution. I. Potentiometric Titration of Aqueous Dye Solution and Calculation of Ionization Constant of Dye

By Mitsuo MURAMATSU

(Received April 30, 1958)

Up to the present, work has been accumulated on the interaction of substances in aqueous solution with surface films of amphipathic compounds spread upon it[1]. Among these, the effect of solute in a substrate solution on monolayers spread on it was extensively studied and many interesting results were obtained. For instance, a remarkable expansion and solidification have been reported for surface film such as stearic acid or octadecylamine due to such a substance cobalt, aluminum, thorium, or alginate ion dissolved in the substrate upon which the film was spread[2,3]. Marked increase in film thickness and wettability were also reported when a built-up film of stearic acid was immersed in solutions containing these ions[4].

All these phenomena seem to show that the nature of the film is profoundly affected by ions which interlink with each other and are taken up by the film. To confirm this, further contributions will be made from the study of interaction between mono- or multilayers of amphipathic compounds and aqueous solutions of dyes, since the latter compounds have a tendency to form micelles or aggregates in an aqueous medium[5] and, as a result, they behave sometimes as if they were macromolecular electrolyte. It will be presumed, however, that the association of molecules is dominantly affected by the degree of ionization of dissociating groups in the dye molecule, which can be calculated from their respective ionization constants and pH of the medium. In spite of such a significance of ionization constant, little was successfully attempted to determine it for usual dyes. This may be due to the failure of the application of simple method of pK determination[6], while a more rigorous method gives values of low reliability in spite of an elaborate calculation as in

1) A general review, cf. M. Muramatsu, T. Sasaki and R. Matuura, *Reports, Kyūshū Univ., Chem. Sect.*, **2**, 47 (1955).

2) T. Sasaki and R. Matuura, This Bulletin, **24**, 274 (1951); T. Sasaki and M.Muramatsu, ibid., **29**, 35 (1956); J. H. Schulman et al., *Trans. Faraday Soc.*, **46**, 475 (1950); **50**, 1128, 1131, 1139 (1954), etc.

3) E. G. Cockbain and J. H. Schulman, *Trans. Faraday Soc.*, **35**, 716 (1939); T. Sasaki and M. Muramatsu, This Bulletin, **26**, 96 (1953).

4) M. Muramatsu and T. Sasaki, This Bulletin, **25**, 21, 25 (1952); A. Inaba, ibid., **26**, 43 (1953).

5) E. L. Valko, "Colloid Chemistry" edited by J. Alexander, Vol. 6, Reinhold Publ. Corp., New York, (1946), p. 594; see also W. Pauli and F. Lang, *Monath. Chem.*, **67**, 159 (1936).

6) e. g., L. Michaelis, Physical Method of Organic Chemistry, Part II, in "Technique of Organic Chemistry", edited by A. Weissberger, Interscience Publ., Inc., New York, (1949), p. 1712.

the case of polybasic acid[7].

The present paper describes an improved method of evaluating the dissociation constant of polybasic acid and polyacidic base, as well as the results obtained for several dyes.

Method of Calculation

The following three assumptions are made for calculating ionization constants of ionizable groups in the dye molecule.

(i) Dye, OH^-, Cl^-, Na^+, etc., in the titration mixture are so dilute that the concentration instead of the activity is used in calculation.

(ii) Volume of titration mixture is the sum of the volume of the solution of dye and of titrating acid or alkali.

(iii) As for the concentration of the carbonate ion, the effect due to the second ionization constant is small enough to be neglected in the pH region of the present experiment.

Now we show the case of titrating a solution of chrysoidine, one of the diacidic bases, with a solution of sodium hydroxide or hydrochloric acid. Ionization constants, K_1 and K_2, for this dye are defined by

$$\left.\begin{aligned} R^{2+} &\rightleftarrows R^+ + H^+ : \ K_1 = [H^+][R^+]/[R^{2+}] \\ R^+ &\rightleftarrows R + H^+ : \ K_2 = [H^+][R]/[R^+] \end{aligned}\right\} \quad (1)$$

where R, R^+, and R^{2+} are the dye molecules possessing zero, one, and two ionized amino groups, respectively, and [ ] denotes the concentration of inscribed species. Total concentration, C, of the dye is then expressed by

$$C = [R] + [R^+] + [R^{2+}] \quad (2)$$

We now introduce a parameter f different from Schwarzenbach's[7], defined by

$$f = 2[R^{2+}] + [R^+] \quad (3)$$

Then, from the condition of electrical neutrality

$$f = [Cl^-] + [OH^-] + [HCO_3^-] - [Na^+] - [H^+] \quad (4)$$

Substituting 1 and 3 into 2 we obtain

$$\left(\frac{f}{C}\right) + \left(\frac{f}{C} - 1\right)\frac{[H^+]}{K_2} + \left(\frac{f}{C} - 2\right)\frac{[H^+]^2}{K_1K_2} = 0 \quad (5)$$

In the case of aluminon, one of the acidic dyes, the following relations result;

$$\left.\begin{aligned} K_1 &= [H^+][R^-]/[R] \\ K_2 &= [H^+][R^{2-}]/[R^-] \\ K_3 &= [H^+][R^{3-}]/[R^{2-}] \end{aligned}\right\} \quad (6)$$

$$C = [R] + [R^-] + [R^{2-}] + [R^{3-}] \quad (7)$$

The solution of this dye is titrated with hydrochloric acid, so the parameter f is given by

$$f = -3[R^{3-}] - 2[R^{2-}] - [R^-] = [Cl^-] + [OH^-] + [HCO_3^-] - [NH_4^+] - [H^+] \quad (8)$$

Substituting 6 and 8 into 7 we have

$$\left(\frac{f}{C} + 3\right) + \left(\frac{f}{C} + 2\right)\frac{[H^+]}{K_3} + \left(\frac{f}{C} + 1\right)\frac{[H^+]^2}{K_2K_3} + \left(\frac{f}{C}\right)\frac{[H^+]^3}{K_1K_2K_3} = 0 \quad (9)$$

For the titration of a solution of such an amphoteric dye as methyl orange or tropäolin OO with hydrochloric acid solution, we obtain eventually

$$\left(\frac{f}{C} + 1\right) + \left(\frac{f}{C}\right)\frac{[H^+]}{K_2} + \left(\frac{f}{C} - 1\right)\frac{[H^+]^2}{K_1K_2} = 0 \quad (10)$$

with f being given by the equation 4 and K_1 and K_2 by

$$\left.\begin{aligned} K_1 &= [H^+][R^\pm]/[R^+], \text{ for sulfonic group} \\ K_2 &= [H^+][R^-]/[R^\pm], \text{ for basic group} \end{aligned}\right\} \quad (11)$$

where $R^\pm$ denotes the zwitterion form of dye molecule.

Similar treatment applied to Congo red, possessing two amino and two sulfonic groups in a molecule, leads us to the expression

$$\left(\frac{f}{C} + 2\right) + \left(\frac{f}{C} + 1\right)\frac{[H^+]}{K_4} + \left(\frac{f}{C}\right)\frac{[H^+]^2}{K_3K_4} + \left(\frac{f}{C} - 1\right)\frac{[H^+]^3}{K_2K_3K_4} + \left(\frac{f}{C} - 2\right)\frac{[H^+]^4}{K_1K_2K_3K_4} = 0 \quad (12)$$

with the parameter f determined by 4 and K's by

$$\begin{aligned} K_1 &= [H^+][R^{\pm+}]/[R^{2+}], \text{ for first sulfonic group} \\ K_2 &= [H^+][R^{2\pm}]/[R^{\pm+}], \text{ for second sulfonic group} \\ K_3 &= [H^+][R^{\pm-}]/[R^{2\pm}], \text{ for first amino group} \\ K_4 &= [H^+][R^{2-}]/[R^{\pm-}], \text{ for second amino group} \end{aligned} \quad (13)$$

7) G. Schwarzenbach et al., *Helv. Chim. Acta*, **30**, 1303, 1798 (1947); **32**, 1175 (1949).

From the assumption (ii), $[Cl^-]$, $[Na^+]$ or $[NH_4^+]$ in equation 4 or 8 can be calculated from the initial concentration, C_0, and volume, V_0, of dye solution, as well as initial concentration, C_0', and the volume, V_t, of the solution of acid or alkali added. Further, from the assumption iii

$$[HCO_3^-] = (K_c + [H^+]_0)([H^+]_0 - [OH^-]_0)/K_c$$

and we have also

$$[OH^-] = K_w/[H^+]$$

where K_w and K_c are the ionic product of water and first dissociation constant of carbonic acid, respectively, and $[H^+]_0$ and $[OH^-]_0$ are the concentration of H^+ and OH^-, respectively, in conductivity water into which dye is dissolved. Therefore we can calculate the parameter f from equations 4 or 8. C in equations 5, 9, 10 or 12 can also be calculated from the initial concentration of dye used. Thus, putting the numerical value of f/C for given $[H^+]$ into the equations 5, 9, 10 or 12 enables the evaluation of K's for each dye.

Experimental

Material.—Dyes used were of chemically pure grade and were further purified by a special method, e. g., salting-out[8] for Congo red and methyl orange and reduction-precipitation[9] for aluminon, as the case requires. Although dye suffered hydrolysis occasionally during purification, the purification was continued until the titration curve became unaltered by the subsequent recrystallization. Actually, the successive recrystallization proved able to shift the titration curve of aminazobenzene hydrochloride, as shown in Fig. 1. Color of the solution also changed gradually from red to yellowish-red with the repeated recrystallization. These facts clearly show a detachment of hydrogen chloride from dye base. Assuming no impurity contained in dye, the amount of the acid detached can be calculated from the elemental analysis of the dye. Table I

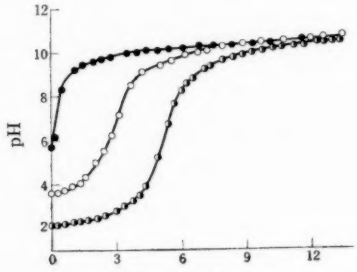
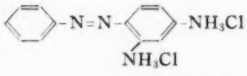
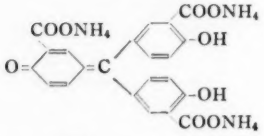
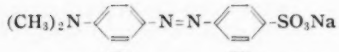
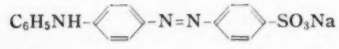


Fig. 1. Effect of recrystallization of aminazobenzene hydrochloride upon the titration curve: $[NaOH] = 1.25 \times 10^{-3}$ mol./l.; $[dye] = 2.0 \times 10^{-4}$ mol./l.; volume of dye solution = 50.00 cc.
◑ recrystallized 3 times
○ recrystallized 6 times
● recrystallized 9 times

TABLE I
MOST PROBABLE COMPOSITION OF DYES USED

Name	Constitution	Type	Occurence, %	Type	Occurence, %
Chrysoidine		R^+Cl^-	98.06	$R^{2+}2Cl^-$	1.94
Aluminon		$R^{3-}3NH_4^+$	26.84	$R^{2-}2NH_4^+$	73.16
Methyl orange		R^-Na^+	80.97	$R^\pm$	19.03
Tropäolin OO		R^-Na^+	78.31	$R^\pm$	21.69
Congo red		$R^{2-}2Na^+$	46.03	$R^\pm - Na^+$	53.97

8) C. Robinson and H. A. T. Mills, *Proc. Roy. Soc.*, **A131**, 576 (1931).

9) N. Caro, *Ber.*, **25**, 939 (1892).

shows the most probable degree of detachment of such an acid or a base for samples used in the experiment. The calculation of composition in the table is based on the elemental analysis of carbon, hydrogen, nitrogen and sodium (for amphoteric dyes) or chlorine (for basic dye). The molar concentration of dye solution was calculated taking into account this detachment of acid or base.

Hydrochloric acid, twice distilled in an all-grand glass apparatus, and sodium hydroxide purified by alcohol were used as titratingagents and standardized with perchloric acid solution. Conductivity water to be used often suffered a slight lowering of pH presumably owing to the dissolution of carbon dioxide, the content of which was calculated as mentioned in the previous section.

Procedure. — Exactly 50 cc. of ca. 2×10^{-4} mol./l. of dye solution was titrated with hydrochloride acid or sodium hydroxide of ca. 1.25×10^{-3} mol./l. under mechanical stirring of 400 rpm and pH change was traced by means of pH meter (Shimadzu Model GU, using GL-6 glass electrode), which was calibrated with buffer solutions of two different pH values. The error introduced in the measurement of pH was estimated to be within ±0.02. Reading of pH was taken after equilibrium was attained for successive titration which was completed within 10 hrs. after the preparation of dye solution. It was confirmed in the preliminary experiment that the effect of aging of dye solution upon the titration curve could be eliminated by leaving it alone for at least 24 hrs.

All experiments were carried out at room temperature of 20 ± 5°C.

Results

Fig. 2 shows the value of pH of the solution of methyl orange titrated with hydrochloride acid. The concentration of each ion species, except for dye ions, in the solution could be calculated from these pH values and from given conditions of $[Na^+]=1.64\times10^{-4}$ g. ion/l. at $V_t=0$,

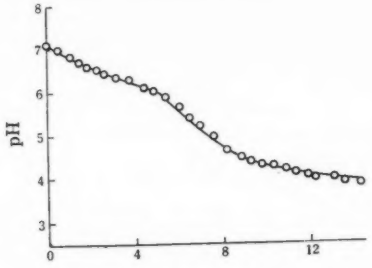


Fig. 2. V_t~pH relationship for methyl orange: $C_0=2.03\times10^{-4}$ mol./l.; $C_0'=1.25\times10^{-3}$ mol./l.; $V_0=50.00$ cc.; ○, observed; ——, calculated by Eq. 10 assuming $K_1=2.75\times10^{-4}$ and $K_2=3.22\times10^{-7}$.

$[Cl^-]=1.25\times10^{-3}$ g.ion/l. for the initial concentration of titrating solution, $[H^+]_0=2.29\times10^{-7}$ ($pH_0=6.64$), $K_w=1.02\times10^{-14}$, and $K_c=3.5\times10^{-7}$. Table II shows the typical examples of these terms and f/C with varying V_t.

In applying these data to 10, we were hardly able to evaluate simultaneously K_1 and K_2 successfully from the graphical plotting of $\left(\frac{f}{C}\right)[H^+]\Big/\left(\frac{f}{C}+1\right)$ against $-\left(\frac{f}{C}-1\right)[H^+]^2\Big/\left(\frac{f}{C}+1\right)$. This was mainly due to the fact that $[H^+]$ changed widely from 10^{-7} to 10^{-4}, as V_t changed from 1 to 13 cc. Therefore a conventional approximation was made to determine K's. Since it was roughly estimated from Fig. 2 that K_2 was nearly of the order of 10^{-7} while K_1 was larger than 10^{-5}, the last term of left side of (10) was considered to be negligible in the region of $[H^+]\approx10^{-7}$. That is, we can safely ignore the reaction

Table II

Concentration of various ion species in the solution and f/C for the titration of methyl orange

V_t	C	$[H^+]$	$[Na^+]$	$[Cl^-]$	$[OH^-]$	$[HCO_3^-]$	f	f/C
1.0	1.99×10^{-4}	1.45×10^{-7}	1.61×10^{-4}	2.45×10^{-5}	0.69×10^{-7}	2.15×10^{-7}	-1.38×10^{-4}	−0.694
2.0	1.95 〃	2.63 〃	1.58 〃	4.81 〃	0.38 〃	1.73 〃	−1.10 〃	−0.565
3.0	1.91 〃	4.27 〃	1.55 〃	7.02 〃	0.23 〃	1.37 〃	−0.85 〃	−0.445
4.0	1.88 〃	6.61 〃	1.52 〃	9.62 〃	0.15 〃	1.06 〃	−0.56 〃	−0.298
5.0	1.85 〃	1.07×10^{-6}	1.49 〃	1.14×10^{-4}	0.10 〃	0.75 〃	−0.36 〃	−0.194
6.0	1.81 〃	1.95 〃	1.46 〃	1.34 〃	0.05 〃	0.46 〃	−0.14 〃	−0.0774
7.0	1.78 〃	6.03 〃	1.44 〃	1.55 〃	0.02 〃	0.17 〃	+0.05 〃	+0.0281
8.0	1.75 〃	1.66×10^{-5}	1.41 〃	1.72 〃	0.00 〃	0.06 〃	+0.14 〃	+0.0800
9.0	1.72 〃	3.47 〃	1.39 〃	1.91 〃	0.00 〃	0.00 〃	+0.17 〃	+0.0988
10.0	1.69 〃	4.90 〃	1.37 〃	2.08 〃	0.00 〃	0.00 〃	+0.22 〃	+0.130
11.0	1.66 〃	7.08 〃	1.34 〃	2.26 〃	0.00 〃	0.00 〃	+0.21 〃	+0.127

$R^{\pm}+H^+ \rightarrow R^+$ in this region, since $f=[R^+]-[R^-]$ is negative in this region. Thus we have a linear relationship between $-(f/C)[H^+]$ and $(f/C)+1$ in the region of $[H^+] \leq 5\times10^{-7}$ as shown in Fig. 3, from which we obtain $K_2=3.30\times10^{-7}$. Putting this value into 10 and plotting

$$-\left(\frac{f}{C}-1\right)\frac{[H^+]^2}{K_2} \text{ against } \left(\frac{f}{C}+1\right)+\left(\frac{f}{C}\right)\frac{[H^+]}{K_2},$$

as shown in Fig. 4, we obtain $K_1=2.73\times10^{-4}$. Successive approximation not neglecting the last term gave limiting values

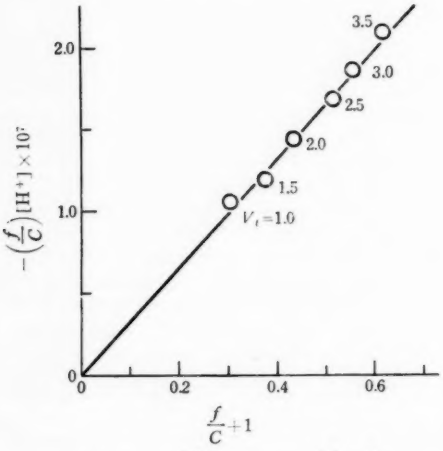


Fig. 3. $-\left(\frac{f}{C}\right)[H^+]$ vs. $\left(\frac{f}{C}+1\right)$ for methyl orange.

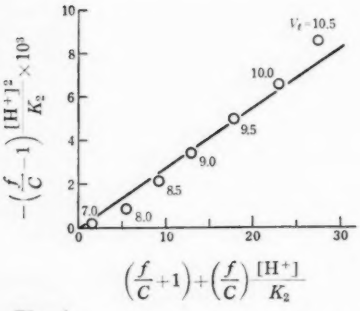


Fig. 4. $-\left(\frac{f}{C}-1\right)\frac{[H^+]^2}{K_2}$ vs. $\left(\frac{f}{C}+1\right)+\left(\frac{f}{C}\right)\frac{[H^+]}{K_2}$ for methyl orange.

of $K_1=2.75\times10^{-4}$ and $K_2=3.22\times10^{-7}$. Substitutions of these values back into 10 give f/C for given $[H^+]$ which in turn gives V_t. V_t~pH relationship thus obtained is expressed as a full line in Fig. 2. A fairly good coincidence is seen between observed and calculated values in this figure.

The open circles in Fig. 5 denote the observed values of pH of the solution of tropäolin OO titrated with hydrochloric acid. Similarly to the case of methyl orange, we obtained the dissociation constants, K_1 and K_2, defined by 11. The final values were $K_1=5.60\times10^{-4}$ and $K_2=2.98\times10^{-7}$. Again, the dependence of pH upon V_t is reproduced by putting these values into 10. The full curve in Fig. 5 represents the V_t~pH relationship thus obtained.

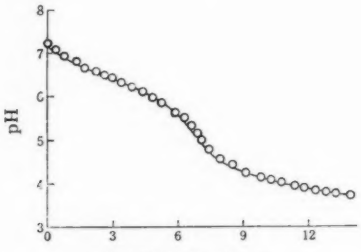


Fig. 5. V_t~pH relationship for tropäolin OO: $C_0=2.08\times10^{-4}$ mol./l., $C_0'=1.25\times10^{-3}$ mol./l., $V_0=50.00$ cc; ○, observed; ——, calculated by Eq. 10 assuming $K_1=5.60\times10^{-4}$ and $K_2=2.98\times10^{-7}$.

Solution of chrysoidine was titrated separately with the solutions of hydrochloric acid and sodium hydroxide, since the greater part of starting sample of dye is of R^+ form. The result is shown in Fig. 6. Putting the experimental data into equation 5, we obtained $K_1=4.84\times10^{-4}$ and $K_2=7.09\times10^{-6}$. V_t~pH relationship reproduced from these values of K's is shown as a full curve in Fig. 6.

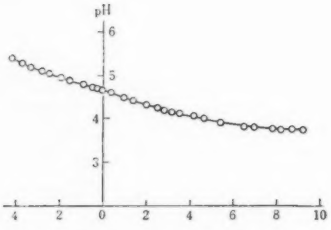


Fig. 6. Titration curve for chrysoidine: $C_0=2.01\times10^{-4}$ mol./l., C_0' of HCl$=1.25\times10^{-3}$ mol./l., C_0' of NaOH$=1.58\times10^{-3}$ mol./l., $V_0=50.00$ cc.; ○, observed; ——, calculated by Eq. 5 assuming $K_1=4.84\times10^{-4}$ and $K_2=7.09\times10^{-6}$.

Fig. 7 shows the titration curve of aluminon. When we followed the method of calculation mentioned above to equation 9, $K_3=6.08\times10^{-8}$ and $K_2=1.595\times10^{-5}$ were obtained. In calculation of K_1, however, plotting of $(f/C)\ [H^+]^3/K_2K_3$ against

$$\left(\frac{f}{C}+3\right)+\left(\frac{f}{C}+2\right)\frac{[H^+]}{K_3}+\left(\frac{f}{C}+1\right)\frac{[H^+]^2}{K_2K_3}$$

was unsuccessful, owing to considerable fluctuation of the plot. If we reproduce the $V_t\sim$pH relationship assuming $K_2=1.595\times10^{-5}$, $K_3=6.08\times10^{-8}$ and K_1 being

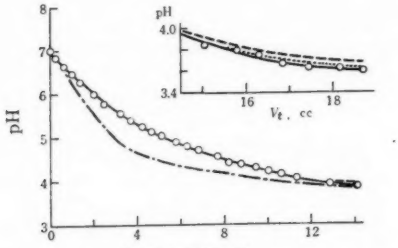


Fig. 7. Titration curve for aluminon: $C_0=2.06\times10^{-4}$mol./l., $C_0'=1.44\times10^{-3}$mol./l., $V_0=50.00$ cc.; ○, observed; curves, calculated by Eq. 9 assuming $K_2=1.595\times10^{-5}$, $K_3=6.08\times10^{-8}$ and $K_1\approx1$ (——), 10^{-2} (------) or 10^{-3} (– – –); semidotted curve, based on high-molecular electrolyte.

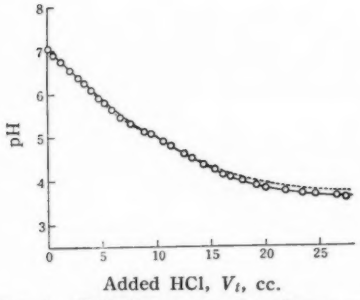


Fig. 8. Titration curve for Congo red: $C_0=2.03\times10^{-4}$ mol./l., $C_0'=1.25\times10^{-3}$ mol./l., $V_0=50.00$cc.; ○, observed; -----, calculated by Eq. 12; ——, calculated by Eq. 16.

either of 1, 10^{-2} or 10^{-3}, we obtain three corresponding curves differing from one another in the tail parts of titration curves reproduced, as seen in the figure inserted in Fig. 7. Thus, when we take into account the experimental error in determining pH, we can say no more than $K_1=10^{-2}$ or larger.

Titration curve for Congo red is shown in Fig. 8. From these data we obtained the following values of K's using equation 12;

$$K_4=9.59\times10^{-8}$$
$$K_3=7.19\times10^{-6}$$
$$K_2=1.82\times10^{-4}$$

Again, we failed to plot and calculate the value of K_1, similar to the case of aluminon. Therefore we could merely evaluate K_1 to be 10^{-1} or larger. In Fig. 8 the dotted curve represents the calculated values of V_t for given pH using these experimental values for K's. We can see some deviations between calculated and observed values of pH for given V_t at the tail part of the titration curve.

Discussion

In Table III are summarized the values of pK's determined for each ionizable group in the dye used, except for Congo red. It should be noticed that the ionization constants are defined by 1, 6 or 11 and, therefore, that the stronger acid is of the smaller pK, while the stronger base is of the larger pK.

TABLE III
pK'S OF IONIZABLE GROUPS IN THE DYE

Dye	Acid group	Basic group
Chrysoidine	——	$pK_1=3.32$ $pK_2=5.21$
Aluminon	$pK_1\leq2$, $pK_2=4.80$ $pK_3=7.22$	——
Methyl orange	$pK_1=3.56$	$pK_2=6.49$
Tropäolin OO	$pK_1=3.25$	$pK_2=6.53$

It is well known that, when the second acid group of the same kind is introduced in the molecule having one acid group, the ionization constant of the former becomes smaller than that of the latter, while the value of the latter is slightly affected by the addition of the former. A typical example of this is seen in the case of dicarboxylic acids[10]. When we apply this principle to the dibasic dye, the pK values of chrysoidine are explained as those expected from the ionization constant, $pK=2.91$ as defined by 1, for aminazobenzene[11]. By means of the

10) B. Adell, "Über die Elektrolytische Dissoziation von Dicarbonsäuren in Alkalichloridlösungen", Lund, (1938).
11) R. C. Farmer and F. J. Warth, *J. Chem. Soc.*, **85**, 1726 (1904).

methods of titration and spectrophotometry, Woislawaski[12] has also reported $pK_2=5.3$ for an amino group in chrysoidine in aqueous solution.

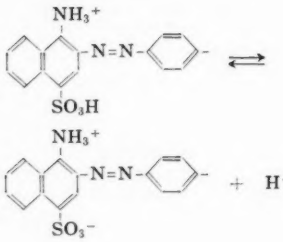
Substitution of amino group by sulfonic group makes little difference in pK_2 between chrysoidine and methyl orange or tropäolin OO. It is hardly believed that the ionization constant of an amino group in the dye is remarkably affected by dimethylation or phenylation, both of which show little effect upon the ionization constant of aniline[13]. It is further noted that pK_1 of methyl orange or tropäolin OO is of nearly the same value as that, $pK=3.28$ at 20°C, for sulfanilic acid[14].

It seems to be premature to ascribe the deviation of calculated pH value from the observed one at the tail part of the titration curve of Congo red, as seen in Fig. 8, to a mere experimental error. Actually the deviation is larger than that due to the pH measurement and exhibits a systematic tendency; namely, the calculated value is always larger than the observed one. Now, let us make a tentative explanation of this deviation which is ascribed to the special nature of Congo red in aqueous solution. If we assume two sulfonic groups, not distinguishable from each other with regard to their ionization, in the molecule, then we can treat Congo red similarly to a high-polymer electrolyte. We can safely assume this, since two sulfonic groups are both strongly ionizing and are far apart from each other. At least, such a consideration would be valid for the region where titrating hydrochloric acid reacts with these acid groups. The high-molecular or micellar nature of Congo red, suggested by the data of ultracentrifuge[15] and diffusion[5], in aqueous medium would also support this consideration.

From this standpoint we shall take the following K's, instead of those defined by 13, for Congo red as a high polymer-like substance;

$$\left.\begin{aligned} K_1=K_2&=[H^+][R^{2\pm}{}_{1/2}]/[R^{2+}{}_{1/2}]\\ [R^{\pm}{}_{1/2}]&=2[R^{2\pm}]\\ K_3&=[H^+][R^{\pm-}]/[R^{2\pm}]\\ K_4&=[H^+][R^{2-}]/[R^{\pm-}] \end{aligned}\right\} \quad (14)$$

where $R^{2\pm}{}_{1/2}$ and $R^{2+}{}_{1/2}$ express the unit segments in ionizing molecule, as appears in



Consequently

$$\left.\begin{aligned} C&=[R^{2-}]+[R^{\pm-}]+[R^{2\pm}]+1/2[R^{2+}{}_{1/2}]\\ f&=[R^{2+}{}_{1/2}]-[R^{\pm-}]-2[R^{2-}]\\ &=[Cl^-]+[OH^-]+[HCO_3^-]\\ &\quad-[Na^+]-[H^+] \end{aligned}\right\} \quad (15)$$

Substituting 15 into 14 we obtain eventually,

$$\left(\frac{f}{C}+2\right)+\left(\frac{f}{C}+1\right)\frac{[H^+]}{K_4}+\left(\frac{f}{C}\right)\frac{[H^+]^2}{K_3K_4}+\left(\frac{f}{C}-2\right)\frac{[H^+]^3}{K_2K_3K_4}=0 \quad (16)$$

Application of the successive approximation to 16 leads us to the following result;

$$K_4=1.252\times10^{-7}$$
$$K_3=4.75\times10^{-6}$$
$$K_1=K_2=1.83\times10^{-3}$$

Table IV shows pK values of respective ionizing groups in Congo red for two equations on which the calculation is based.

TABLE IV

pK VALUES FOR CONGO RED

Equation used	Acid group	Basic group
(12)	$pK_1\leq1$ $pK_2=3.74$	$pK_3=5.14$ $pK_4=7.02$
(16)	$pK_1=pK_2=2.74$	$pK_3=5.32$ $pK_4=6.90$

In Fig. 8 the full and dotted curves express the $V_l\sim$pH relationship reproduced using equations 16 and 12, respectively. A better coincidence of equation 16 shows the validity of the above treatment. Applying a similar treatment to aluminon, we obtained $K_1=K_2=2.5\times10^{-4}$ and $K_3=6.18\times10^{-8}$ for this dye but we failed to confirm the coincidence between observed and calculated values in the range of $V_l>$

12) S. Woislawski, *Proc. Soc. Exp. Biol. Med.*, **79**, 390 (1952); *J. Am. Chem. Soc.*, **75**, 5201 (1953).
13) Landolt-Börnstein, "Physikalisch-Chemische Tabellen", 5th ed., **2**, 1159, second suppl., 1093 (1923).
14) R. O. MacLaren and D. F. Swinehardt, *J. Am. Chem. Soc.*, **73**, 1822 (1951).
15) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Clarendon press, Oxford, (1940), p. 310.

12 cc., as shown as semi-dotted curve in Fig. 7.

Various methods have been presented to determine the ionization constant. Of the methods, spectrophotometry seems hardly applicable for a polyionizing substance. In contrast with this, the potentiometric method is, in principle, applicable to any substance. However, we must solve a rather complicated equation which is liable to produce an appreciable error for a larger ionization constant appearing in the higher-order term of the equation[7]. In order to minimize the error and to facilitate the calculation, it is important to adopt a suitable parameter. The present method of successive approximation is convenient in evaluating the ionization constants of a polyionizing acid or base.

Summary

Change of pH of aqueous dye solution was traced by titrating with the solution of hydrochloric acid or sodium hydroxide. From these data, ionization constants for each ionizing group in chrysoidine, methyl orange, tropäolin OO and aluminon were calculated, making use of equations based on the law of mass action with a specially-selected parameter. The validity of the ionization constants thus determined were confirmed by reasonable coincidence of the titration curves obtained by calculation using these data with those observed, over the wide range of pH.

In the case of Congo red, however, it seemed better to treat it as a high-molecular electrolyte. Ionization constants calculated by an equation based on such a treatment explain the experimental result better than those based on the treatment similar to the case of other simple dyes.

In conclusion, the author wishes to express his sincere thanks to Professor T. Sasaki for his guidance and encouragement during this experiment. A part of the cost of this research has been defrayed from the Scientific Research Expenditure of the Ministry of Education.

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Studies on the Interaction of Surface Films with Solute in Solution. II. Sorption of Dye on the Built-up Film of Stearic Acid and Barium Stearate

By Mitsuo MURAMATSU

(Received April 30, 1958)

Knowledge of the surface interaction between amphipathic compounds in the film state and dyes in aqueous solutions contributes considerably not only to the understanding of the mechanism of the dying processes of fiber, the solubilization of dye, the physiological action of bile and other living dye, etc., but also to the knowledge of the substance in its two-dimensional state. From this standpoint the effect of aqueous dye solution on the monolayer of stearic acid[1], cetyl acetate, synthetic fibers, and other polar-nonpolar compounds[2] were studied. Sobotka[3], studying the adsorption of physiologically active substance including porphyrin derivatives upon the built-up film of stearic acid and protein, pointed out an important role of molecular structure of adsorbate in increasing the film thickness and affecting the wettability. It should be further noticed in the case of dye substance that such a type of interaction may be affected

1) R. Matuura, This Bulletin, **24**, 282 (1951).

2) M. M. Allingham, C. H. Giles and E. L. Neustädter, *Discussion Faraday Soc.*, No. **16**, 92 (194).

3) Sobotka, "Recent Advances in Surface Chemistry and Chemical Physics", Science Press Printing Co., Lancaster, (1939), p. 54.

considerably by the nature of dye ions, especially by the balance of anionic and cationic charges, as was pointed out for the interaction of a number of dyes and anionic and cationic detergents in an aqueous medium[4].

In connection with these, the present author intends to study the action of dye ion upon the built-up film of stearic acid and related substances, under varying pH of aqueous dye solution. It may be expected furthermore that such a study may contribute to the knowledge of surface interaction between metal ion and fatty acid, which was reported by Sasaki and Matuura[5], Schulman et al.[6], and Sasaki and the present author[7,8].

Experimental

Reagent.—The stearic acid was the same as that used in a preceding experiment[7]. The dyes were all Takeda's or Merck's reagent grade, except for benzoazurin G (C. I. No. 502)* and benzopurpurin 4B which were kindly supplied by Mr. M. Fujimoto of Mitsubishi Kasei Co. Of the dyes used Congo red, methyl orange and crystal violet were thoroughly purified by repeated recrystallization[9]. Other dyes were used without any special purification, since it was confirmed in the preliminary experiments that the successive purification of Congo red and crystal violet resulted in little change in the sorbed amounts of dyes.

Method.—Monolayer of stearic acid spread on a substrate of pH 4 was successively transferred by means of Langmuir-Blodgett technique[10] onto a highly polished slide of stainless steel, so as to obtain a base echelon. Then a half part of it was immersed for thirty seconds into an aqueous solution of the dye 10^{-4} mol./l. varying in its pH, while the other half was immersed into a control solution of the same pH without dye. The surface of dye or control solution was swept by two barriers prior to immersion. After washing the film with distilled water the changes in thickness of both parts of the echelon were measured by means of the simplified optical method[11], assuming the refractive index of dye to be the same as that of the base material. Calculation was based on the fact that spacing and refractive index of stearic acid in the multilayer were 22.9 Å and 1.482, respectively[10]. The difference, Δ, in changes of thickness thus calculated between two halves of the slide was taken for the sorbed amount. In the case of the solution of crystal violet, night blue (C. I. No. 731), benzoazurin G, or phloxine (C. I. No. 778), each of them forming an elastic film, the down- and up-trip of slide sometimes resulted in the building-up of an adsorbed layer at the air-solution interface. For such a dye, therefore, all experiments were carried out for different times of immersion during thirty seconds, against which the amount of Δ was plotted. A typical example is shown in Fig. 1. True values of the sorbed amount were assumed to be obtained by extrapolation of the curves to zero time of immersion.

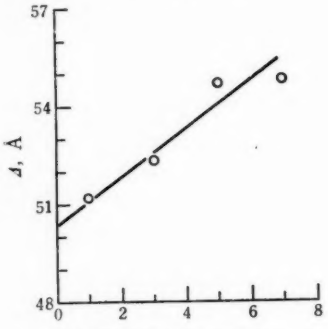

Fig. 1. Effect of immersion upon the apparent amount, Δ, of sorption of crystal violet: pH 6.5.

Experiments were also carried out using the echelon of barium stearate, instead of stearic acid, for the interaction with Congo red and crystal violet. In this case the built-up film was obtained by transferring the monolayer of stearic acid spread on an aqueous substrate containing barium chloride onto the slide. Spacing and refractive index were assumed to be 24.4 Å and 1.462[10], respectively, for this case. All experiments were carried out for 10^{-4} mol./l. of dye solutions, pH of which was varied merely using redistilled hydrochloric acid and aqueous ammonia solution and its value was determined using Beckman's glass electrode.

Results

Acid Dyes.—Fig. 2 shows the effects of pH of dye solution on the amount of aluminon (triammonium aurintricarboxylate) sorbed on the built-up film of stearic acid and on the degree of ionization of three carboxyl groups in the dye molecule. The latter values were calculated using $pK_1 \leq 2$, $pK_2 = 4.80$ and $pK_3 = 7.22$ for each

4) G. S. Hartley, *Trans. Faraday Soc.*, **30**, 444 (1934).
5) T. Sasaki and R. Matuura, This Bulletin, **24**, 274 (1951); R. Matuura, ibid., **24**, 277 (1951).
6) J. H. Schulman et al., *Trans. Faraday Soc.*, **46**, 475 (1950); **50**, 1128, 1131, 1139 (1954); *Discussion Faraday Soc.*, No. **16**, 158 (1954).
7) M. Muramatsu and T. Sasaki, This Bulletin, **25**, 21, 25 (1952).
8) T. Sasaki and M. Muramatsu, ibid., **29**, 35 (1956).
9) C. Robinson and H. A. T. Mills, *Proc. Roy Soc.*, **A131**, 576 (1931).
* Abbreviation according to "Color Index", edited by F. M. Rowe, Society of Dyers and Colourisis, Bradford, First edition, (1924).
10) K. B. Blogett and I. Langmuir, *Phys. Rev.*, **51**, 964 (1937), etc.
11) T. Tachibana, *J. of Japanese Biochem. Soc.*, (*Seikagaku*), **20**, 8 (1948).

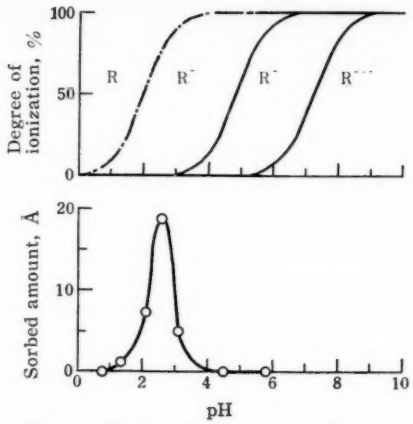

Fig. 2. Effect of pH on the degree of ionization of aluminon and on its amount sorbed on the built-up film of stearic acid.
Semi-dotted curve represents the lower limit of degree of ionization for a given pH, with regard to the reaction, $R \rightleftarrows R^- + H^+$.

groups[12]. They decreased with decreasing pH of the solution, while a maximum sorption was observed at pH from 2 to 3, where the majority of dye molecules were of singly ionized carboxylic acid.

The pH dependence of the amount of phloxine, scarlet red and benzoazurin G sorbed is shown in Fig. 3. All these dyes showed a large amount of sorption in an acidic medium, though there exist some differences in details. Phloxine, one of the triphenylmethane dyes having one carboxyl and one phenolic group, showed a maximum amount of sorption in the range of pH from 3 to 4, where we usually observed a faint turbidity in the bulk and a remarkable rigidity in the surface of the dye solution. Precipitation of dye was evident when pH decreased beyond this range. In the case of benzoazurin G, neither the maximum point of sorption nor the precipitation of dye was observed throughout pH range investigated. The amount sorbed on the film proved to increase with decreasing pH for benzoazurin G. Scarlet red, having both one phenolic and one sulfonic group in the molecule corresponding to a split-in-half structure of benzoazurin G, showed a small amount of sorption at pH less than 3.

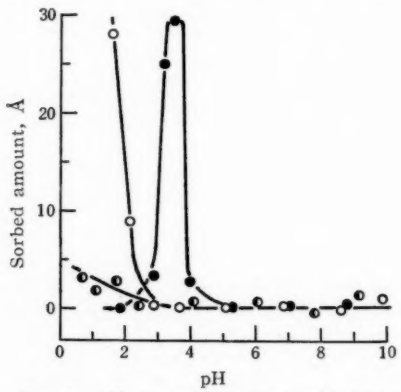

Fig. 3. pH vs. sorbed amount of phloxine (●), scarlet red (◐) and benzoazurin G (○).

Basic Dyes.—In Fig. 4 full curves represent the pH dependence of the amount

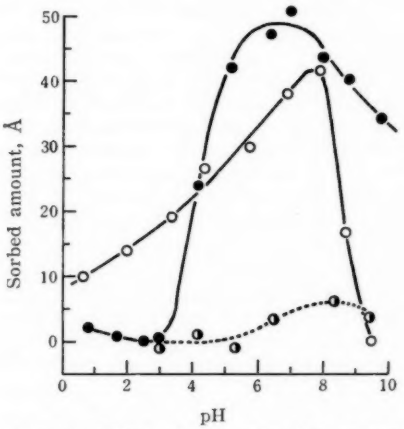

Fig. 4. Effect of pH upon the amount of dye sorbed on the built-up film: ○, night blue on stearic acid; ● crystal violet on stearic acid; ◑, crystal violet on barium stearate.

of crystal violet and night blue sorbed on the built-up film of stearic acid. Maximum sorption was observed in the range of pH from 6 to 8 for both dyes where we often experienced a remarkable wetting of a paraffined wall of the tray by a heavily foaming solution of dye. In the same figure the dotted curve shows the effect of pH upon the amount of crystal violet sorbed on the built-up film of barium stearate. When we compare this curve with the corresponding full curve, it is evident that the use of barium stearate instead of stearic acid resulted in a remarkable diminution of interaction with dye.

12) M. Muramatsu, This Bulletin, **31**, 864 (1958).

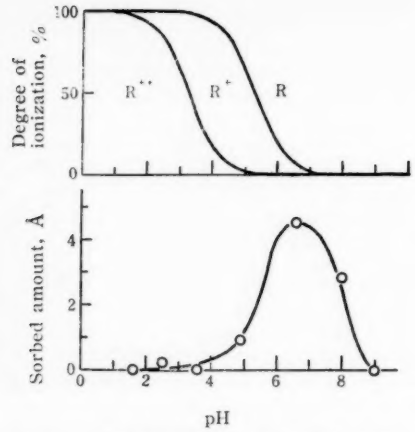

Fig. 5. pH dependence of degree of ionization and of amount of chrysoidine sorbed on the built-up film of stearic acid.

Curves for ionization and sorption of chrysoidine are shown in Fig. 5. The former curves were obtained assuming $pK_1=3.32$ and $pK_2=5.21$[12]. It can be seen in the figure that the maximum of sorption lies in the range of pH from 6 to 7 where one of the amino groups is ionized to the extent of less than 20% while the other is in an entirely non-ionized state.

Amphoteric Dyes.—In Figs. 6 to 8 are shown the results for the dyes possessing both the acidic and the basic groups. Ionization curves for acid and basic groups in methyl orange, tropäolin OO (C. I. No. 143) and Congo red are calculated from all pK values which have been reported in the preceding paper[12].

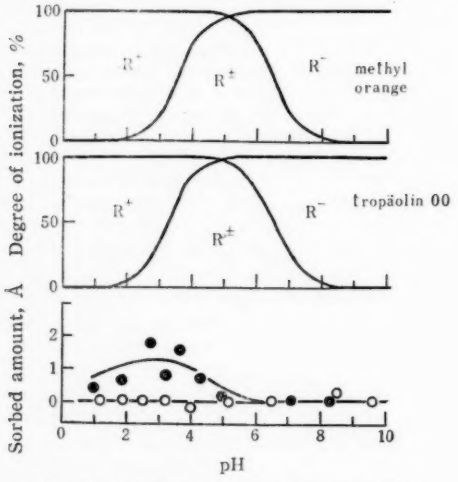

Fig. 6. pH dependence of degree of ionization and of sorbed amount of tropäolin OO (●) and methyl orange (○).

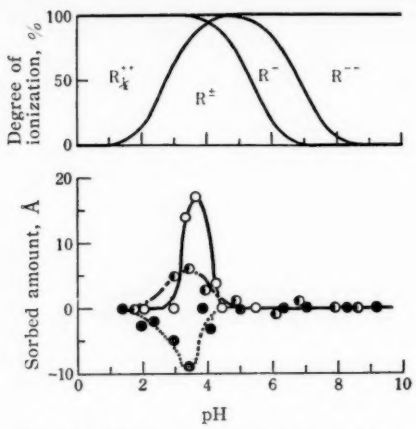

Fig. 7. pH dependence of degree of ionization and of amount of Congo red sorbed on the built-up film of stearic acid (○) and of barium stearate, with (◐) or without (●) the conditioning by the solution of aluminum chloride.

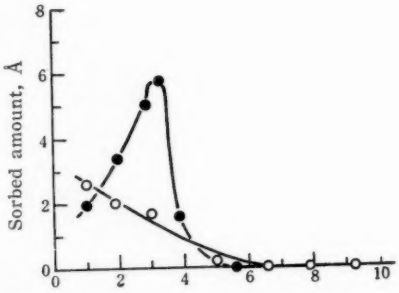

Fig. 8. pH vs. sorbed amount of benzopurpurin 4B (●) and trypan red (○).

Of the dyes investigated, tropäolin OO, Congo red and benzopurpurin 4B showed a maximum of sorption on the film of stearic acid in pH range characteristic of each substance, while no measurable sorption was observed for methyl orange in the pH range from 1 to 10. It can be seen furthermore in Figs. 6 and 7 that pH ranges for maximum sorption of tropäolin OO and Congo red were roughly in agreement with the isoelectric region where the majority of dye molecules were in zwitterion form.

Striking results were obtained for the interaction of Congo red with barium

stearate in the film. When the echelon was immersed in an aqueous solution of dye, the pH of which was in the range of 2 to 4, we recorded the decrease in thickness of the echelon, which is shown as a dotted curve in Fig. 7. This might be ascribed to the dissolution of molecules of barium stearate into the solution of dye, which could be seen as the indication of the interaction between the dye and stearate. Similar phenomena have been reported in the case of interaction between serum protein and built-up film of stearic acid[13] or barium stearate[14]. In the other pH range, however, no change was observed in the thickness of the echelon. On the other hand, whenever the film was once conditioned with an aqueous solution of aluminum chloride, the subsequent immersion in the dye solution of pH 2 to 4 always resulted in an increase in the thickness of the film, as is shown by a brokenline in the same figure.

It is seen in Fig. 8 that benzopurpurin 4B has a maximum in pH range for sorption on the built-up film of stearic acid. In contrast with this, trypan red (C. I. No. 438) seemed to be increasingly sorbed with decreasing pH of the solution, this behavior being observed in the case of an acid dye such as benzoazurin G or scarlet red.

Discussion

In our former reports[7], the sorption of metal ion on the built-up film of stearic acid could be explained by the ionic interaction. This has been confirmed[8] by the experiments using radioactive $^{89(90)}Sr$ and ^{60}Co. It was pointed out, however, in the case of the monolayer of octadecylamine[15] that the behavior of the surface film depended markedly upon the molecular structure of the solute in aqueous subphase, so that we should take account not only of the ion-ion interaction but also such an interacting force as ion-dipole, dipole-dipole, hydrogen-bonding and/or van der Waals' attraction when the molecule of solute was complicated in structure. From such a point of view, Matuura[1] explained the expansion of the monolayer of stearic acid spread on aqueous solution of dye by an attraction between the dye and the stearic acid, involving ionic and van der Waals' forces.

In the present experiment the action of acid dyes on the built-up film seems to show that van der Waals' attraction plays an important role in the marked adsorption. It would be likely that the decreasing negative charges of both acid dye and stearic acid with decreasing pH of the medium may weaken the repulsive force acting between the dye molecules themselves as well as between the molecules of dyes and stearic acid. On the other hand dipole-dipole and van der Waals' attraction are considered to be largely independent of the pH of the medium. Thus the decreasing repulsion may cause the increasing sorption of dyes on the built-up film. These are the cases with benzoazurin G and scarlet red. The fact that the sorbed amount of the former is much greater than that of the latter, as shown in Fig. 3, may suggest a large contribution of van der Waals' attraction to the amount of sorption, since the latter is of the split-in-half structure compared with the former.

If, however, ionic repulsion is reduced too much, we can expect such a large aggregates that they are removed from the region of the interfacial film. It is therefore probable that the maximum amount of sorption is observed, if the molecular weight of dye is sufficiently great and the pH of the medium is in a range where the molecules of dye retain an appropriate amount of charge. Fig. 2 offers a typical example for such an effect of ionization upon the sorbed amount of aluminon, the molecular weight of which has the order of magnitude of 800. Dye is presumably in a colloidal state when the pH of the solution is in the range of optimum sorption. Such an explanation would also be made for the case of phloxine, the molecular weight of which is of the same order of magnitude as that of aluminon. From the studies on the velocity of ultrasonic wave in the bulk solution[16] and on the rheological properties of the surface[17] of aqueous solution of phloxine, it has been pointed out that dye molecules are presumably in a state of colloidal aggregate at the pH regions beyond which they precipitate. It may be reasonable therefore to ascribe the appearance of maximum

13) I. H. Sher and H. Sobotka, *J. Collold Sci.*, **10**. 125 (1955).
14) T. Tachibana. K. Fukuda and K. Hamaguchi, This Bulletin, **24**, 155 (1951).
15) E. G. Cockbain and J. H. Schuman, *Trans. Faraday Soc.*, **35**, 716 (1939); T. Sasaki and M. Muramatsu, This Bulletin **26**, 96 (1953).
16) T. Yasunaga and T. Sasaki, To be published in future.
17) H. Kimizuka, This Bulletin, **26**, 33 (1953).

sorption to the formation of colloidal aggregates resulting from the balance between attractive force and ionic repulsion.

A similar explanation can be made for the interaction of basic dyes with stearic acid in the film, results of which are shown in Figs. 4 and 5. A large contribution of van der Waals' force to the interaction may be suggested when we consider the pH dependence of the degree of ionization together with that of the amount of chrysoidine sorbed on the film. It should be noticed, however, that chrysoidine retains still a small amount of positive charge even at pH 6.5 where the maximum interaction is observed, while stearic acid is considered to have a slight negative charge when it is in the state of monolayer spread on distilled water of the same acidity[18]. Therefore the ionic attraction between dye and stearic acid which may exaggerate the sorption should not be neglected. A similar explanation was made for the interaction between stearic acid in the built-up film and metal ion in aqueous solution[7]. Remarkable solidification of the surface film of fatty acid spread on an aqueous crystal violet[1,19] could also be explained from this point of view. This idea is further supported by the fact that the built-up film of barium stearate takes up a smaller amount of crystal violet than that of stearic acid does.

Somewhat complicated results are obtained in the case of amphoteric dyes, which are shown in Figs. 6 to 8. It can be seen in general that the maximum sorption is observed near the isoelectric region of dye. This is indeed the case for tropäolin OO and Congo red. The isoelectric region of benzopurpurin 4B may presumably be around pH 3, where the built-up film of stearic acid takes up the maximum amount of dye from the solution. The exceptional behavior of trypan red would be caused by its negative charge due to five sulfonic groups overwhelming the positive charge due to two amino groups. It might be expected therefore that the maximum sorption could be attained if the solution was rendered much more acidic than in the present experiment.

The majority of molecules of amphoteric dye are considered to be in zwitterion form in the isoelectric region, so that they might exist as colloidal aggregates or micelles differing in size and shape, according to their physico-chemical properties[20]. The molecules in the aggregates would be bound to each other by dipolar attraction, hydrogen-bonding and/or van der Waals' force. In the other pH region the ionic repulsion due to the same sign of charge would suppress the association of dye molecules. Such a presumption may be valid from the results of rheological study on the surface of aqueous dye solution[12], in which it was seen that a film was most coherent for an amphoteric dye when a solution is in an isoelectric region. Such a mechanism for the interaction between dye molecules themselves may also serve to explain the attraction between the built-up film and the aggregate of dye. This presumption is supported by a greater amount of sorption in a respective isoelectric pH range for a larger molecule of dye. Non-sorption of methyl orange may be explained by the smallest molecular weight. An exception is observed however in the relation between Congo red and benzopurpurin 4B. As would be seen in Figs. 7 and 8, the built-up film of stearic acid takes up less benzopurpurin 4B than Congo red, in spite of the molecule being larger by two methoxy groups in the case of the former dye. This may come from the less favorable conditions of forming the aggregate for the latter, presumably owing to the steric hindrance by methoxy groups facing each other in the diphenyl group in the molecule. From the measurement of diffusion velocity of dye, it has been reported[21] that the former dye showed a degree of association of about 9 in 0.01 M sodium chloride solution while the latter had a degree of association of only 6 in the same medium.

It is well known[7,10] that the nonpolar surface of built-up film of barium stearate is rendered polar when it is conditioned by the solution of such cations as copper, aluminum and thorium. This has been explained by the overturning of the molecules in the outermost layer of the film. In connection with this, it is of interest that Congo red interacts with the built-up film of either stearic acid or barium stearate, irrespective of the pre-treatment by the solution of aluminum chloride.

18) J. Glazer and M. Z. Dogan, *Trans. Faraday Soc.*, **49**, 448 (1953).
19) E. Heymann and A. Yoffe, ibid., **38**, 408 (1942).
20) W. Pauli and F. Lang, *Monatsh. Chem.*, **67**, 159 (1936).
21) E. L. Valko, "Colloid Chemistry", edited by J. Alexander, Vol. 6, Reinhold Publ. Corp., New York, (1946), p. 601.

Similar results were obtained for the wettability of the surfaces of various substances including pure paraffin when they were immersed into the solution of Congo red[22]. Again, van der Waals' attraction plays an important role in such a type of interaction. Precisely speaking, however, the possibility of dipolar attraction interacting between dye and film molecules seems to be open to discussion. This is supposed from the fact that the optimum pH range shifts to a more acid position than the pH region where the molecules of dye are almost in zwitterion form, as shown in Fig. 7. At any rate, it would be reasonably deduced that the same attractive force by which the dye molecules are bound to each other is also mainly responsible for the interaction between dye and built-up film molecules, and thus for the change in thickness of the film.

Summary

The amounts of dyes sorbed on the built-up films of stearic acid and of barium stearate were measured, under varying pH of the dye solutions into which the films were immersed. It has been observed in general that the amount of sorption was greater when the molecular weight of dye became greater. Nonionic attraction such as van der Waals' force, which is considered to be a cause of interaction between dye molecules themselves, and aggregate formation among them may also be responsible for the interaction between dye and built-up film. Such an interaction may be favored by the decreasing ionic charges of the same sign existing on dye molecule and film.

Acid dyes proved to be increasingly sorbed with decreasing pH until the aggregates of dye grew so large that they fell from the interface as a precipitate. The difference in detailed behaviors for sorption of several acid dyes could be explained by the difference in the strength of repulsive force of dye ions, suppressing the attraction, as well as of van der Waals' force which was considered to be largely affected by molecular weight.

In the case of basic dyes, ionic attraction is additionally taken into account to explain their sorption on the film of stearic acid. This has been suggested by the correlation between the degree of ionization and sorbed amount of chrysoidine, and supported by the fact that the built-up film of barium stearate took up less crystal violet than that of stearic acid did.

In the case of the sorption of amphoteric dyes, a maximum was observed in general in a pH range which is roughly in agreement with an isoelectric region of dye. Tentative explanations have been made for this phenomenon, taking account of a change in the dissolved state of dyes.

The author expresses his hearty thanks to Professor T. Sasaki for his kind guidance in the course of this study and also to Professor R. Matuura of Kyūshū University for his discussion on this work. The cost of this research has been defrayed from the Scientific Research Expenditure of the Ministry of Education given to Professor Sasaki.

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22) A. Inaba, unpublished work.

Studies on the Interaction of Surface Films with Solute in Solution. III. Effect of Dissolved Dyes upon the Monolayer of Octadecylamine

By Mitsuo MURAMATSU

(Received June 24, 1958)

In a preceding paper[1] the action of dissolved dye on the built-up film of stearic acid and barium stearate was reported. There it has been shown that the maximum amount of solute taken up by the film was observed for dye solution in a specific pH range where the remarkable change of dissolved state in bulk[2], as well as the change of rheological properties in surface[3], was observed for dye solution. Various kinds of behavior in such a type of interaction could be explained chiefly by the balance of the attractive and repulsive forces acting between film materials and dyes, when we take account of their ionized as well as dissolved state. This was in general accord with the experimental results on the effect of the aqueous dye solutions not only upon the nature of the monolayer of stearic acid[4] but also upon the wettability of its solid surface[5].

In order to check the validity of such reasoning it has been attempted in the present work to investigate the interaction between several dyes in aqueous solution and octadecylamine monolayer spread on it.

Experimental

Of the samples of dyes used in the preceding work[1], chrysoidine, crystal violet, scarlet red, phloxine (C. I. No. 778*), trypan red (C. I. No. 438), and benzoazurin G (C. I. No. 502) were chosen also for the present experiment, because of the typical behavior of each in the interaction with stearic acid. Each of these dyes was dissolved in water and a stock solution of 10^{-2}mol./l. was made, which was diluted to 10^{-4}mol./l. prior to use as a substrate solution of the monolayer. To minimize the disturbing effects of coexisting ions for dye, pH of the solution was controlled simply by the solutions of hydrochloric acid and ammonia, both of which are twice distilled in an all-ground glass apparatus. The hydrogen ion concentration of this solution was determined using Beckman's glass electrode.

The octadecylamine was the same as that used in a former experiment[6]. It was spread from benzene solution onto the aqueous solution of dye. As we actually used bicarbonate of amine, calculation of the cross-sectional area, A, per molecule was based on the nitrogen content of material which was determined for original samples by micro Dumas method. Surface pressure, F, was measured by means of the modified hanging-plate method[7].

All experiments were carried out at room temperature.

Results

Effect of pH of Substrate Water upon the Monolayer of Amine Spread on It.— It was pointed out by the present author[6] that poor reproducibility was observed for the $F\sim A$ curves of the monolayers of octadecylamine when they were spread on the surface of distilled water. This could be ascribed chiefly, in agreement with the opinion of the other investigators[8,9], to differences in the content of carbon dioxide in water, aging of the film, the velocity of compression, etc. Such an effect was observed also in the present experiment in which pH of substrate water was widely changed over the range of 1 to 10. But $F\sim A$ curve of the monolayer fluctuated only within the hatched zone shown in Fig. 1, though the solution was rendered sufficiently acidic.

In Fig. 2 open circles represent the area, A_0, of octadecylamine molecule at $F=2$ dyn./cm. for varying substrate pH. If we take account of the ambiguity mentioned above, we could not express the $A_0\sim$pH relationship more precisely than as a hatched zone shown in the figure. In

1) M. Muramatsu, This Bulletin, **31**, 871 (1958).
2) T. Sasaki and T. Yasunaga, to be published.
3) H. Kimizuka, This Bulletin, **26**, 33 (1953).
4) R. Matuura, ibid., **24**, 282 (1951).
5) A. Inaba, unpublished work.
* Abbreviation according to "Color Index" edited by F. M. Rowe, First edition, (1924).
6) T. Sasaki and M. Muramatsu, This Bulletin, **26**, 96 (1953).
7) T. Sasaki, *J. Chem. Soc. Japan, Pure Chem. Sec. (Nippon Kagaku Zassi)*, **62**, 796 (1941).
8) J. Marsden and J. H. Schulman, *Trans. Faraday Soc.*, **34**, 748 (1938).
9) W. C. Bigelow, E. Glass and W. A. Zisman, *J. Colloid Sci.*, **2**, 563 (1947); E. J. Hoffman, G. E. Boyd and A. W. Ralston, *J. Am. Chem. Soc.*, **64**, 498 (1942).

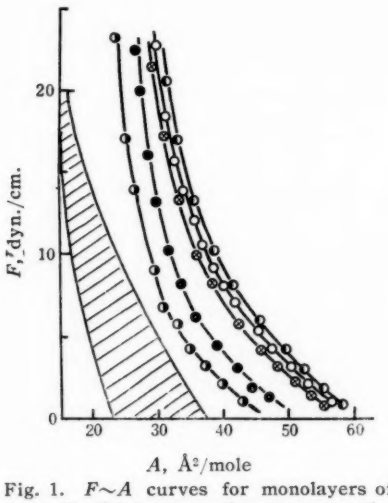

Fig. 1. $F\sim A$ curves for monolayers of octadecylamine spread on aqueous solution of crystal violet under varying substrate pH: hatched zone expresses the limit of fluctuation of $F\sim A$ curves in the control (without dye) experiments for varying substrate pH.

- ◑ pH 2.9
- ● 5.5
- ⊗ 6.1
- ◐ 7.1
- ○ 9.8

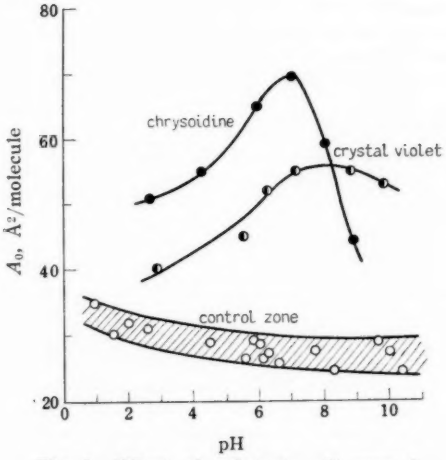

Fig. 2. Effect of substrate pH upon A_0 of octadecylamine spread on aqueous solution of basic dye and on distilled water (control experiment).

general the monolayer showed a tendency of expansion on an acidic substrate. This made a contrast with the case of stearic acid spread on an alkaline substrate[10] and, as assumed by the other authors[8,10], might be attributed to the ionic repulsion due to the ionization of the monolayer.

Monolayer of Amine on the Solution of Basic Dye.—In Fig. 1 are shown the $F\sim A$ curves for the monolayers of octadecylamine spread on the solutions of crystal violet of varying acidity. It is seen in the figure that the $F\sim A$ curve shifts gradually towards right, showing the increasing expansion of the monolayer with increasing pH of substrate. Such an effect of pH upon the nature of the monolayer is reproduced in Fig. 2, in which pH dependence of A_0 of octadecylamine is shown for the cases of crystal violet and chrysoidine. For both dyes we observed characteristic pH ranges for maximum expansion of the monolayer.

Monolayer of Amine on the Solution of Acid Dye.—In the presence of phloxine in the substrate, we observed a marked effect of pH upon F—A curve for the monolayer of octadecylamine, as illustrated in Fig. 3. It can be seen in the figure that the remarkable expansion and increase in compressibility of the film were observed when pH of the substrate was

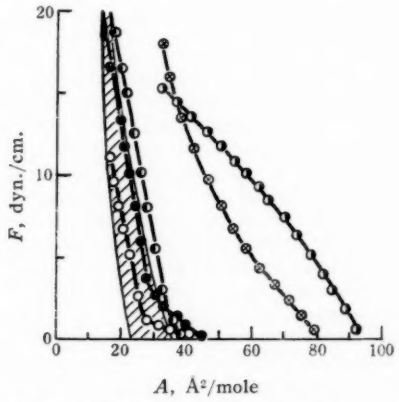

Fig. 3. $F\sim A$ curves for monolayers of octadecylamine spread on aqueous solutions of phloxine, under varying pH: hatched zone expresses the limit of fluctuation of $F\sim A$ curves for the control experiments.

- ◐ pH 1.7
- ◑ 2.9
- ⊗ 3.5
- ● 4.1
- ○ 7.9

10) N. K. Adam, "The Physics and Chemistry of Surfaces", Oxford University Press, London, (1937), p. 71.

around 3~3.5. In the case of scarlet red, however, little change was observed in the cross-sectional area of amine even if pH of the substrate solution was small. The compressibility of the monolayer of amine was not affected by the presence of this dye. Fig. 4 shows the A_0~pH

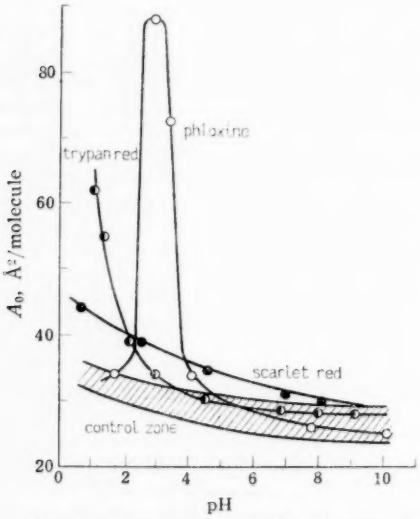

Fig. 4. Effect of substrate pH upon A_0 of octadecylamine on aqueous solution of acid dye.

relation for these dyes, together with for trypan red which made the monolayer expand at pH less than 4 but did not affect its compressibility throughout the whole pH range investigated. It has been pointed out in a preceding paper[1] that trypan red, in spite of its molecule containing five sulfonic and two amino groups, should be regarded rather as an acid dye from the viewpoint of pH dependence of the dye solution.

A somewhat different feature was observed in the case of benzoazurin G. When a fresh surface of a solution of pH 1.7 was compressed by barriers, we recorded a remarkable increase in surface pressure, F, owing to its adsorption to the surface of its own. Though the value of F fluctuated considerably for a given area, σ, of the surface bounded by two barriers, F~σ curve lies always in the zone A in Fig. 5. A more exaggerated feature of F~σ relationship was observed for the monolayer of octadecylamine spread on this solution. In Fig. 5, the hatched zone B and C express the limit of fluctuation in F~σ relationship for the monolayer of

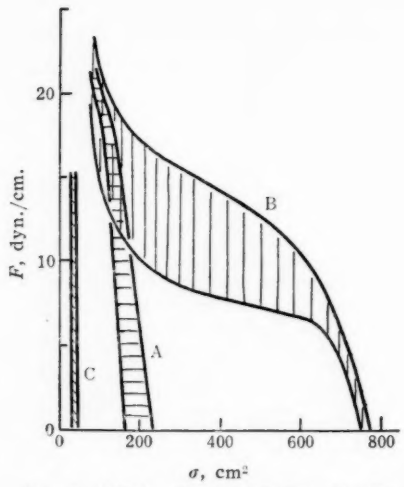

Fig. 5. Limit of fluctuation of F~σ curves: (A) for the surface of solution of benzoazurin G, (B) for the monolayer of 1.29×10^{16} molecules of octadecylamine spread on the substrate containing and (C) lacking the dye. Substrate pH 1.7.

1.29×10^{16} molecules of octadecylamine spread on a substrate of the same pH, containing or lacking benzoazurin G. As is seen in the figure, behavior in the low pressure region exhibited a typical example of the profound effect of benzoazurin G upon the monolayer of amine spread on it. Now, let us compare the surface area, σ_0, at $F=2$ dyn./cm. for

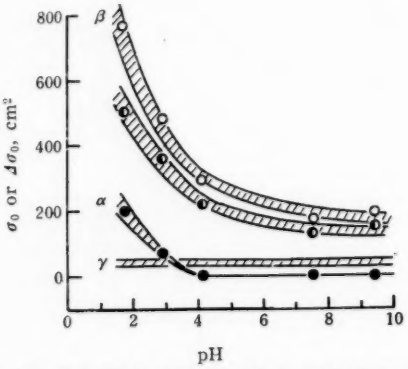

Fig. 6. pH dependence of σ_0 and of $\Delta\sigma_0$ at $F=2$ dyn./cm.: zone α, pH dependence of σ_0 for adsorbed dye film of its own; β and γ, for the monolayer of 1.29×10^{16} molecules of octadecylamine spread on the substrate containing or lacking, respectively, this dye.

zone B with those for A and C. A similar procedure was applied also for the experiment, varying pH of substrate. The results are illustrated in Fig. 6 in which the velues of σ_0's are plotted against pH. It can be seen that the amounts of σ_0 for zone β (amine+dye) are generally far greater than the sum of those for zone α (amine alone) and γ (dye alone). Such behavior is favorably expessed in terms of the deviation, $\Delta\sigma_0$, from the additivity in σ_0 of the monolayers of amine and the adsorbed dye film of its own and it is plotted against substrate pH in the same figure. It is surprising that the amount of $\Delta\sigma_0$ for around pH 1.7 was evaluated to be 500 cm.^{2}, the figure corresponding to 380 Å^2/molecule of amine. Again, we can see a similar effect as observed in the case of scarlet red or trypan red.

Discussion

It would be reasonable to assume that in the present results an expansion of the monolayer of octadecylamine spread on an aqueous solution of dye comes from an interaction between the monolayer and the dye. Then, the more profoundly the interaction arises between octadecylamine and a certain dye, the more remarkably the monolayer would be expected to expand, apart from the quantitative discussion on the amount of expansion.

Adam[11] observed an expansion of the monolayer of octadecylamine spread on a solution of simple organic acid and explained the phenomenon by ion—ion attraction between the amine and the acid. Such a consideration was verified by means of the mono- and multilayer techniques applied for the interaction between octadecylamine in film state and such simple acids as oxalic, malonic, succinic and adipic acid in an aqueous solution[6]. It has been shown[12], however, that we should also take account of an additional contribution of van der Waals' attraction to the expansion of monolayer of amine, which interacts with organic compound of large molecular weight such as thapsic acid or *pp'*-dihydroxydibenzyl in an aqueous substrate. Because of the dye molecule being larger than these compounds, the present results can hardly be explained at all unless we take account of various forces of interaction. Actually, the behavior of basic dye in alkaline region, as shown in Fig. 2, can not be explained by ion—ion attraction. In this case, ionic repulsions between dye molecules themselves and between the molecules of amine and the dye are considered to decrease with increasing pH of the substrate. Therefore increase of substrate pH may cause an increasing tendency of dye to be bound to the monolayer of amine by the attraction different from ion—ion interaction. This would be valid for the region of pH not large enough to form an aggregate of dye, due to the perfect disappearance of the repulsion. Such a consideration has also been made to explain the action of dissolved acid dye upon the mono-[4] or multilayer[1] of stearic acid.

It has been pointed out in a preceding paper[1] that the conditions favorable for the formation of aggregate of dye are generally in accord with those for the enhancement of the surface interaction. Chrysoidine makes the monolayer of amine expand most remarkably, when it was dissolved in the substrate of pH 7 in which the molecules of dye have little ionic charge, as expected from its pK values, i. e., $pK_1=3.32$ and $pK_2=5.21$[13]. In contrast with this, the maximum amount of aluminon, one of the acid dyes, sorbed on the built-up film of stearic acid was observed when pH of the dye solution was around 2~3 where we can expect the effect of ionic repulsion due to the remaining charge[1]. The difference in the molecular weight between chrysoidine (M. W. =250) and aluminon (M. W.=480) may be responsible for the difference in the contribution of van der Waals' attraction to the interaction between dye molecules themselves by which the difference in easiness of aggregation can also be accounted for. However, it should be noticed that the molecules of amine in the monolayer were considered to be in perfectly ionized state as presumed from the experimental data of surface potential[14]. In this case, therefore, we can expect the possibility of an additional contribution of ion—dipole attraction to the expansion of the monolayer.

A similar explanation can also be made for the effect of acid dye upon A_0 or alternatively $\Delta\sigma_0$ of octadecylamine which is shown in Fig. 4 or 6, respectively; namely, decreasing charge of dye with

11) N. K. Adam, *Proc. Roy. Soc.*, **A126**, 526 (1930).
12) E. G. Cockbain and J. H. Schulman, *Trans. Faraday Soc.*, **35**, 716 (1939).
13) M. Muramatsu, This Bulletin, **31**, 864 (1958).
14) J. Glazer and M. Z. Dogan, *Trans. Faraday Soc.*, **49**, 448 (1953).

decreasing pH of the substrate will cause the formation of aggregate of dye with which octadecylamine interacts. If we confine ourselves to this point of view, the expansion of a monolayer may be accounted for by such attractions as dipolar and van der Waals' forces. However, it has been pointed out by means of mono-[4] and multilayer[1] technique, that the effect of basic dye dissolved in alkaline medium upon the surface film of stearic acid was due to the strong interaction being of complex nature, involving not only dipolar and van der Waals' force but also ionic attraction. For the present results, therefore, we had better take into consideration ionic attraction in addition to dipolar or van der Waals' force. At least we can find no objection to such a reasoning for the expansion of octadecylamine monolayer by scarlet red, trypan red and benzoazurin G. Since small pK values are expected for sulfonic groups in the molecules of these dyes[15], an electrostatic attraction may contribute throughout a wide range of substrate pH to the expansion of the monolayer of octadecylamine, the pK of which is reported to be 8.5[14].

It has been shown[2,3] that acid dye formed colloidal aggregates when it was dissolved in aqueous solution of pH being slightly larger than that for distinct precipitation. A typical example was observed in the case of phloxine dissolved in aqueous medium. Actually we observed faint turbidity in this solution when pH was less than 3. Thus the remarkable expansion of the monolayer on substrate of pH 3 can be accounted for by the interaction between the molecules of octadecylamine and the colloidal aggregates of dye. In connection with this, it is interesting to note that the more the monolayer of octadecylamine expands, the more it becomes compressible. This behavior is shown in Fig. 3. The same type of $F \sim A$ curve was observed also for the interaction between stearic acid in the monolayer and cobalt ion in the substrate[16]. This has been explained by the ionized monolayer of stearic acid with which the colloidal aggregate of cobalt ion interacts. Studies on the dissolved state of cobalt ion[17] seemed to support this explanation.

15) N. Andō, "Senryō Kōshitsugaku (Chemistry of Dye Stuffs in Japanese)," Shōkabō, Tokyo, (1948), p. 44.

Summary

The interaction between octadecylamine in monolayer and various dyes in aqueous substrate under varying pH was studied. Such a basic dye as chrysodine or crystal violet remarkably expanded the monolayer when pH of the solution was in a certain range on alkaline side. This has been attributed to the interactions between the molecules of dye themselves and that between dye and octadecylamine. Thus, it is mainly dipolar and van der Waals' attraction, which was considered to be overshadowed by ionic repulsion when substrate pH was in the other region. Additional possibility of ion-dipole attraction due to the remaining charge of the monolayer was discussed in connection with the ionizations of octadecylamine and basic dyes.

In contrast with this, the monolayer expanded remarkably when it was spread on an acidic solution of acid dye. The behavior could be accounted for by the sum of ionic, dipolar and van der Waals' attraction. The possibility of the formation of colloidal aggregate of dye with which octadecylamine interacts has been discussed, in connection with the effect of metal ion in the substrate upon the monolayer of stearic acid.

The author wishes to express his hearty thanks to Professor T. Sasaki for his kind guidance and encouragement throughout this research. His thanks are also due to the Ministry of Education for the Scientific Research Expenditure.

Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, Setagaya-ku, Tokyo

16) T. Sasaki and M. Muramatsu, This Bulletin, **29**, 35 (1956).

17) R. Matuura and H. Kimizuka, This Bulletin, **28**, 668 (1955); T. Sasaki and K. Izawa, unpublished work.

Untersuchungen über die Bildung der Proteinase aus einem Aspergillus awamori-Stamm. I. Faktoren mit Einflüssen über die Bildung der Proteinase

Von Jiro HOSHINO

(Eingegagen am 1. Mai, 1958)

Die proteolytischen Enzyme werden bekanntlich von verschiedenartige Schimmelpilzen in die Nährlösung sezerniert, die deswegen von alters her in den orientalischen Ländern zur Gärungsindustrie verwendet worden sind. Über den gründlichen Bildungsmechanismus der Proteinase aus dem Schimmelpilz ist aber wenig untersucht worden. Es ist daher dem Verfasser ein besonderes Interesse, solche Probleme mittels eines Pilzes *Aspergillus awamori* aufzuklären, das vom Verfasser aus der Erde isoliert wurde und ein starkes Vermögen zur Bildung der "sauren" Proteinasen bietet, von denen der Verfasser eine enzymatische Natur angegeben hat[1].

Die Bildung der Proteinase unter den verschiedenen Züchtungsbedingungen durch das Oberflächenverfahren hat der Verfasser näher studiert[2,3], aber infolge der Mycelentwicklung ist das Versuchssystem in diesem Falle etwas kompliziert und damit wird der wesentliche Bildungsmechanismus des Enzyms schwer aufzuklären.

Verwendet man dagegen das ausgewaschene Schüttelmycel in einem stickstofffreien Milieu, so kann man die oben erwähnte Schwierigkeit leicht beseitigen.

In den letzten Jahren wurden die Bildungsmechanismen der Enzyme von mehreren Forschern, wie z. B. dasselbe der Amylase aus *Bacillus subtilis* von J. Fukuomoto et al.[4] und von M. Nomura[5], der Amylase aus *Asp. oryzae* von O. Tanabe et al.[6] und der Proteinase aus *Asp. oryzae* von H. Shiota et al.[7], mittels des ausgewaschenen schüttelmycels oder der Zellen mitgeteilt.

Um weitere Kenntnisse über den Bildungsmechanismus der "sauren" Proteinase zu erkennen, hat der Verfasser in den vorliegenden Arbeiten das ausgewaschene Schüttelmycel als Versuchsmaterial zur Prüfung unterworfen.

Arbeitsmethode

Gewinnung des ausgewaschenen Schüttelmycels.—Einige Anzahl der Schüttelkolben von 500 ccm Inhalt wurden mit je 80 ccm Nährlösung beschickt, die 10% Rohrzucker, 0.2% Ammoniumnitrat und Monokaliumphosphat, 0.025 % Magnesiumsulfatheptahydrat, 0.002% Mangansulfattetrahydrat, 0.0025% Eisen(III)-chloridhexahydrat und 0.03% Gelatine als Nährsubstrat enthält. Nach dem üblichen Sterilisieren wurden jede Kolben von den etwa 5~10 Tagen Würzeagarkulturen mit Conidiosporen von Stamm Nr. 82—2 geimpft, und danach wurden die Kulturen 72 Stunden lang auf einer Schüttelmaschine mit 130 Hin- und Herbewegungen pro Minute bei 30°C behandelt. Das so sich entwickelnde Mycel wurde dann unter sterilen Bedingungen gemeinsam auf einem groben Filter gesammelt. Das gut abgesaugte und ausgewaschene Mycel wurde in kleine Stücke (1.0 g feuchtes: etwa 0.2 g Trockengewicht) zerschnitten, welche zur Untersuchungen der Bildung der Proteinase in den 50 ccm Versuchslösungen wieder suspensiert, und bei 30°C weiter geschüttelt wurden.

Bestimmung der proteolytischen Aktivität.—Zur Ermittelung der proteolytischen Aktivität hat der Verfasser die Caseinverdauung mittels des Folinschen Reagens nach B. Hagiwara[8] kolorimetrisch bestimmt, wobei die enzymatische Reaktion unter den Bedingungen von 1% Casein, pH=3.2, bei 55°C eine Stunde lang geprüft wurde. Der Wert der proteolytischen Aktivität wurde

1) J. Hoshino und N. Taketomi, *Waseda Applied Chem. Soc. Bull.* (*Waseda Ōyokagakukaihō*), **24**, No. 65 9 (1957).

2) J. Hoshino, *Synopses of Eng. Graduate School of Sci. and Eng. Waseda Univ.* (*Waseda Daigaku Kōgaku Kenkyu Ihō*), **6**, 64 (1957).

3) J. Hoshino, *Memoirs of College of Sci. and Eng., Waseda Univ.* (*Waseda Daigaku Rikogakubu Kiyō*), (im Drucke).

4) J. Fukumoto, T. Yamamoto und D. Tsuru, *J. Agr. Chem. Soc. Japan* (*Nippon Nogei Kagaku Kaishi*), **31**, 421, 429, 506, 510, 545 (1957).

5) M. Nomura et al., *J. Biochem*, **43**, 251, 841 (1956), **44**, 87 (1957).

6) O. Tanabe et al., *J. Agr. Chem. Soc. Japan* (*Nippon Nogei Kagaku Kaishi*), **30**, 596 (1956), **28**, 227 (1954).

7) H. Shiota et al., ebenda, **30**, 724 (1956), **31**, 173 (1957).

8) B. Hagiwara, *Ann. Rep. Fac. Sci. Osaka Univ.*, **2**, 35 (1954).

mit dem Absorptionskoeffizient pro Gramm trockenen Mycels pro 50 ccm Versuchslösung derjenigen mit dem Folinschen Reagens sich färbenden und 0.2 M Trichloroessigsäure löslichen Stoffen, die unter den obigen Bedingungen aus Casein bilden, bei der Wellenlänge von 660 mμ ausgedrückt.

Ergebnisse

1. Bildungsverlauf der Proteinase.—Der Bildungsverlauf der Proteinase aus dem Schüttelmycel wurde unter Verwendung von M/30 KH_2PO_4 (pH=4.8) und des destillierten Wassers als Versuchslösung beobachtet. Die Ergebnisse sind in der Figur 1 dargestellt.

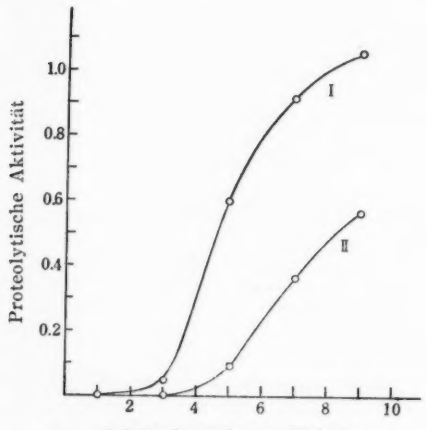

Fig. 1. Bildungsverlauf der Proteinase.
Versuchslösung:
Kurve I, M/30 KH_2PO_4 (pH=4.8)
Kurve II, Destilliertes Wasser

Das Mycel fängt an, wie aus der Figur ersichtlich ist, beim Schütteln mit der stickstofffreien Phosphatlösung nach einer Weile Proteinase reichlich zu sezernieren.

Weiter ist es auch der Fall mit dem Wasser Proteinase zu bilden. Hierbei bleibt aber die Frage noch offen, ob die Proteinase durch das Mycel wirklich *de novo* synthetisch hergestellt wurde oder die innere Proteinase durch das Schütteln ausgelassen wurde. Um diese Frage zu beantworten, hat der Verfasser die Mycelhomogenate und den Auszug des Acetonpulvers des Mycels zur Ermittelung der proteolytischen Aktivität herangezogen. Keine proteolytischen Aktivität wurde darin bestätigt (Tabelle I). Einige metabolischen Inhibitoren, wie Kaliumcyanid, 2, 4-Dinitrophenol und Toluol haben die Bildung der Proteinase verhindert (Tabelle II). Durch die oben angegebenen Tatbestände wurde es festgestellt, dass die Bildung der Proteinase sich nicht das Auslassen der inneren Proteinase, sondern echte Biosynthese durch einen von den genannten metabolischen Inhibitoren verhinderten Prozess stattfindet.

TABELLE I
PROTEOLYTISCHE AKTIVITÄT DES MYCELHOMOGENATES UND DES AUSZUGS

Reaktionsdauer (Min.)*	15	30	60	120
Mycelhomogenate	0	0	0	0
Auszug	0	0	0	0
Kontrolle**	0.39	0.52	0.64	0.71

* Enzymatische Reaktionen wurden unter den Bedingungen von 1% Casein, pH=3.2 bei 55°C ausgeführt.

** Zum Kontrollversuch wurde die Versuchslösung herangezogen, nachdem sie mit dem ausgewaschenen Mycel 20 Stunden lang geschüttelt worden war.

TABELLE II
EINWIRKUNGEN DER METABOLISCHEN INHIBITOREN AUF DIE PROTEINASEBILDUNG

Inhibitoren	Konz.	Proteolytische Aktivität der Versuchslösung, Schüttelungsdauer (Stdn.) 3	5	24
Toluol	10% V/V	0	0	0.06
KCN	1×10^{-2} M	0	0	0.27
	1×10^{-3} M	0	0	2.94
2, 4-DNP*	1×10^{-2} M	0	0	0.09
	1×10^{-3} M	0	0	0.95
Kontrolle	1×10^{-3} M	0	0.24	3.16

* 2, 4-Dinitrophenol

2. Bildung der Proteinase bei der Züchtung und durch das Schütteln des dabei sich entwickelten Mycels.—Als einzelne C-Quelle zur Entwicklung des Pilzes wurden je 10% Saccharose, Glucose, Fructose und Lactose in der Nährlösung zugesetzt und die Anhäufung der Proteinase in der Kulturflüssigkeit nach 3 Tagen und das Bildungsvermögen derselben des so sich entwickelten Mycels beobachtet.

Die Versuche mit dem ausgewaschenen Mycel wurden mittels derjenigen Versuchslösung ausgeführt, die aus M/5-Phosphatpuffergemisch (pH=6.1) und 1% Glucose besteht. Die Resultate sind in der Tabelle III zusammengestellt. Dabei hat es sich ergeben, dass das ausgewaschene Schüttelmycel ein grösseres Vermögen zur Proteinasebildung unter dem Mangel an den

TABELLE III

PROTEINASEBILDUNG DURCH DAS AUSGEWASCHENE MYCEL UND DURCH DAS ENTWICKLUNGSVERFAHREN

	C-Quelle im Nährboden	Oberflächenverfahren	Schüttelverfahren	
		Entwicklungszüchtung*	Entwicklungszüchtung*	Dabei entwickeltes ausgewaschene Mycel**
		7 Tage	3 Tage	24 Stunden
Proteinasebildung (Prot. Akt.)	Saccharose	0.31	0.39	5.29
	Fructose	6.65	0.20	6.53
	Glucose	0.08	0.06	9.70
	Lactose	—	0.82	10.57
Entwicklung (Gewicht des Mycels in mg)	Saccharose	91	560	
	Fructose	913	562	
	Glucose	81	540	
	Lactose	—	116	

* Die Entwicklungsnährlösung steht im Text geschrieben, aber ohne Gelatine für Oberflächenverfahren.

** Die Versuchslösung besteht aus 0.2 M Phosphatpufferlösung und 1% Glucose.

Nährsubstrate als des sich entwickelten Mycels erweist.

3. Proteinasebildungsvermögen des Mycels in den verschiedenen Entwicklungsphasen.—Es ist bekannt, dass sich die physiologische oder biochemische Aktivität der Zellen mit der Dauer der Züchtung ändert. Hinsichtlich dieses Tatbestandes hat der Verfasser das Proteinasebildungsvermögen des Schüttelmycels in den verschiedenen Entwicklungsphasen ermittelt und seine Beziehung zum Gehalt des Mycels an den freien Aminosäuren näher studiert. Die Ergebnisse sind in der Figur 2 illustriert.

Die Anhäufung der Proteinase nach dem zwanzigstündigen Schütteln des Mycels in der 1% glucosehaltigen M/5-Phosphatpufferlösung wurde zur Bestimmung des Proteinasebildungsvermögen ermittelt.

Wie aus der Figur 2 ersichtlich ist, fand eine beträchtliche Änderung des Vermögens des Mycels während seiner Entwicklung statt: je jünger das Mycel ist, desto grösser ist das Vermögen. Dementsprechend sind die reichlichere Mengen der wasserlöslichen Eiweissstoffen und des totalen Stickstoffes im solchen Mycel enthalten. Wenn das Mycel sich kräftig entwickelt, vermindert sich allmählich sein Proteinasebildungsvermögen. Es deutet an, dass es zwischen dem anabolischen Stoffwechsel des sich entwickelnden Mycels und demselben des ruhenden Mycels ein grosser Unterschied gibt, wie von M. Nomura[5] schon darauf hingewiesen wurde.

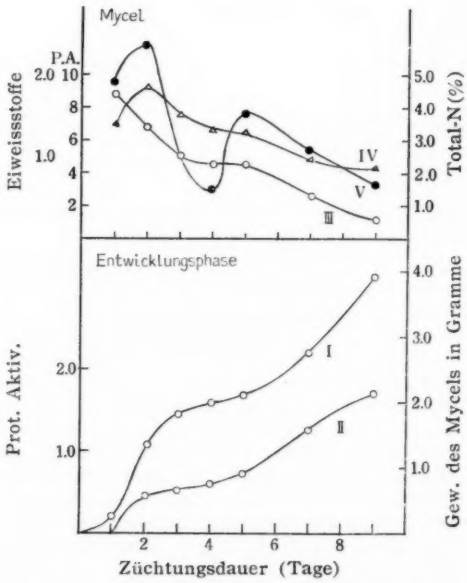

Fig. 2. Änderung des Proteinasebildungsvermögens des Mycels mit der Züchtungsdauer.

Kurve I: Mycelentwicklung
II: Proteinasebildung bei der Züchtung
III: Proteinasebildungsvermögen des Mycels (P. A.)
IV: Stickstoffgehalt des Mycels
V: Wasserlösliche Eiweissstoffe*

* Ausgewaschenes Mycel wurde unter Rückflüsskühler mit Wasser 10 min. dreimal extrahiert, Eiweissstoffe im Extrakt wurden mittels des Folinschen Reagens kolorimetrisch ermittelt.

TABELLE IV

EINFLUSS DER ANAEROBEN BEDINGUNGEN AUF DIE PROTEINASEBILDUNG

	Kontrolle				Anaerobenschütteln								
	Wattebausch				Gummistöpsel				N_2-Einfüllung			Vakuum	
0.2 M-Phosphatpuffer	−	−	+	+	−	−	+	+	−	−	+	+	+
1% Glucose	−	+	−	+	−	+	−	+	−	+	−	+	+
Schluss pH	5.6	1.4	5.9	5.4	5.6	1.8	5.9	5.6	5.6	2.2	5.9	5.8	5.8
Proteolytische Aktivität	4.3	0.7	4.0	4.2	4.0	0.9	3.8	4.4	0.6	1.4	0.8	1.2	0

4. Einfluss des pH auf die Proteinasebildung.—Um den Einfluss des pH auf die Bildung der "sauren" Proteinase zu erkennen, hat der Verfasser die Pufferlösungen von N/10-Phosphat und N/10-Acetat als Versuchslösungen unter den variierenden pH verwendet. Die Ergebnisse sind in der Figur 3 illustriert.

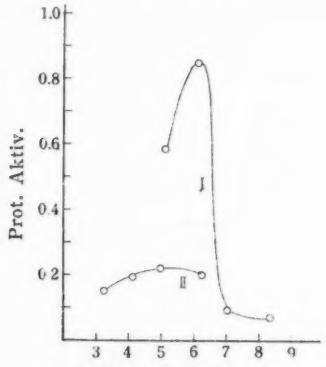

Fig. 3. pH-Abhängigkeit der Proteinasebildung.
Versuchslösung; I, N/10 Phosphatpuffer
II, N/10 Acetatpuffer

Bei den Versuche mit den Phosphatpufferlösungen ergab es sich, dass die Bildung der Proteinase gegen die Änderung des pH äusserst empfindlich ist, deren Optimum bei pH=6.1 liegt. Demgegenüber bleibt die Acetatpufferlösung auf die Bildung der Proteinase ohne Wirkung, die daher durch das Phosphat gar nicht ersetzlich ist. Daraus wurde es festgestellt, dass das Phosphat nicht nur als ein Puffer, sondern auch als ein Hilfsmittel auf die Biosynthese der Proteinase wesentlich einwirkt.

5. Einfluss der Phosphatkonzentration.—Wie im vorigen Paragraph beschrieben, übt die Anwesenheit des Phosphates auf die Bildung der Proteinase eine vorzügliche Wirkung aus, die aber dem Acetat ganz fehlt.

In Berücksichtigung dieser wichtigen Tatsache hat der Verfasser den Einfluss der Phosphatkonzentration der Versuchslösung auf die Proteinasebildung mit dem Anfangswert von pH=6.1 näher studiert. In der Figur 4 sind die Ergebnisse wiedergegeben. Hierbei ist es gezeigt, dass die Änderung der Phosphatkonzentration sehr einflussreich ist und ihr Optimum bei M/30 liegt.

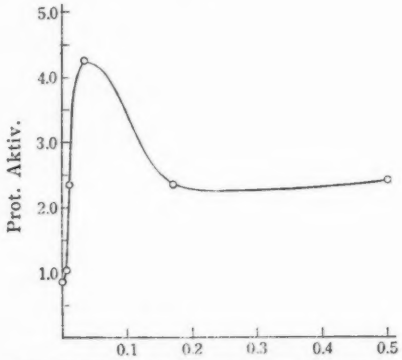

Fig. 4. Einfluss der Phosphatkonzentration.

6. Bedürfnis des Sauerstoffs.—Es wurde von H. Shiota[7] veröffentlicht, dass die Proteinasebildung aus dem ausgewaschenen Mycel des *Asp. oryzae*-Stammes stark gehemmt wird, wenn die Luft in den Schüttelkolben durch Kohlendioxyd, Stickstoff und Wasserstoff ersetzt wird. Identischer Tatbestand hat J. Fukumoto[9] bei den Versuche über die Amylasebildung aus *Bacillus subtilis* wahrgenommen.

Um den Effekt der anaeroben Bedingung bei meinem System in Anbetracht dessen zu erkennen, hat der Verfasser das Mycel je in den Kolben mit dem Gummistöpsel, in der Stickstoffatmosphäre und im Vakuum zum Vergleich mit dem Versuch mit dem Wattebausch geschüttelt. Die Ergebnisse finden sich in der Tabelle IV.

9) J. Fukumoto und T. Yamamoto, *J. Agr. Chem. Soc. Japan* (*Nippon Nogei Kagaku Kaishi*), **31**, 421 (1957).

Diese zeigen, dass Sauerstoff auf die Bildung der Proteinase unentbehrlich einwirkt, dessen Menge aber mit ein wenig genügend ist.

Schlusswort

Es wurde über die Bildungsfaktoren der "sauren" Proteinase aus den ausgewaschenen Schüttelmycel eines Schimmelpilzes *Aspergillus awamori*, das vom Verfasser neulich isoliert wurde und durch das starken Vermögen zur Bildung der "sauren" Proteinese charakteristisch ist, und auf ihre Beeinflussbarkeit durch einige Schüttelbedingungen untersucht.

Zusammenfassend darf es festgestellt werden, dass das Mycel durch einen synthetischen Prozess aus den Aminosäuren die Proteinase bildet, für das die Zusammenwirkung von Sauerstoff und der Energiequelle sowie Phosphat unentbehrlich zu sein scheint. Die pH-Abhängigkeit der Bildung dieses Enzyms ist so bemerkenswert, dass man für die reichliche Bildung mit der konzentrierten Pufferlösung günstigen pH-Wert festhalten muss. Das Optimum liegt bei pH=6.1. Diesbezüglich ist diese Proteinasebildung von derselben bei der Entwicklungszüchtung sehr verschieden, wobei sie im mehr sauren Bereich am günstigsten stattfand, während es noch nicht imstande ist, diesen Unterschied aufzuklären.

Es ist mir ein Bedürfnis, Herrn Professor Dr. N. Taketomi für seine Anregung und stetige Leitung bei dieser Arbeit meinen besten Dank auszusprechen.

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SHORT COMMUNICATIONS

*Chronopotentiometry at Varying Current Density with the Use of Current Controlled Dropping Mercury Electrode**

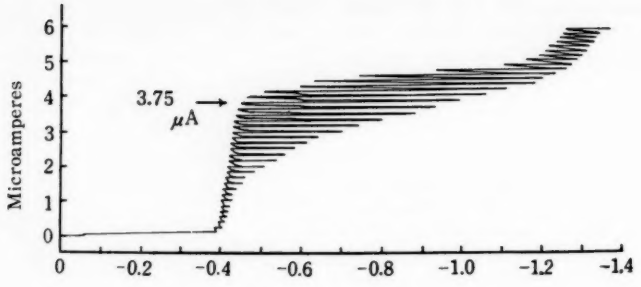
By Masayoshi Ishibashi and Taitiro Fujinaga

(Received May 26, 1958)

The authors have previously introduced a new method for obtaining the polarogram at the dropping mercury electrode with controlled current[1], whose phenomena were mathematically revised by Senda et al.[2] In connection with this study, potential-time curves have been investigated and many interesting phenomena were observed with several electrode reactions. From the stand point of analytical chemistry, it is expected that, with proper control of current, the wave of a more cathodically reducible substance is recorded alone on the chronopotentiogram (potential-time curve), independently, in the presence of large quantities of less cathodically reducible substances. For instance, with a solution of cadmium in hydrochloric acid, a very negative potential corresponding to that of hydrogen deposition is expected at the beginning of the drop growth and then it moves to a positive potential of the cadmium ion—cadmium amalgam system with the decrease in current density due to the increase in surface area, so that a chronopotentiogram of inversed shape to that in the ordinary chronopotentiometry is recorded even in the presence of less cathodically reducible substances e.g. the lead ions. Apparently this characteristic is more effective than any other voltammetric methods hitherto used in trace analysis. Therefore a preliminary report is presented here.

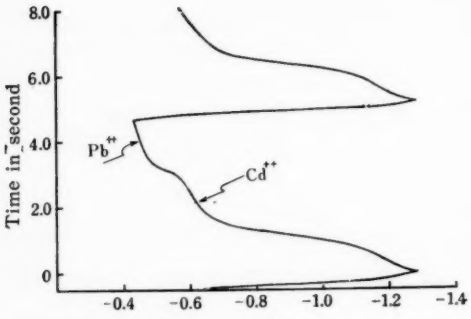
The chronopotentiogram was recorded by the x-y recorder made by Shimadzu Mfg. Co. (Pen speed: 0.8 sec./25 cm.,

1) M. Ishibashi and T. Fujinaga, *J. Electrochem. Soc. Japan (Denki-kagaku)*, **24**, 375, 525 (1956); *Anal. Chim. Acta*, **18**, 112 (1958).
2) M. Senda, T. Kambara and Y. Takemori, *J. Phys. Chem.*, **61**, 965 (1957).
* Presented at the Symposium on Instrumental Methods of Chemical Analysis at Himeji in 1958 sponsored by the Chemical Society of Japan.



Volt vs. S. C. E.

Fig. 1. Current scanning polarogram.
(4×10^{-5}M-Cd^{2+}, 1×10^{-3}M-Pb^{2+}, 0.01%-Polyacrylamide and 0.1 N-HCl)



Volt vs. S. C. E.

Fig. 2. Chronopotentiogram.
(Solution: same as Fig. 1, t=5.26 sec. at i=3.75 μA)

maximum sensitivity: 10 mV./25 cm. and maximum chart speed: 2.0 sec./35 cm.), to which were attached a pre-amplifier of increasing the input impedance for the x-axis and a voltage scanner of changeable velocity for the y-axis. The controlled current was supplied from a DC source composed of a high voltage and a high resistance used in a previous paper[1].

Fig. 1 shows a current scanning polarogram of cadmium ions in the presence of 25 times as many lead ions. Fig. 2 is the corresponding chronopotentiogram for cadmium wave at the current of 3.75 microamperes.

Besides the use of the ordinary dropping mercury electrode for the change of current density, combination of the dropping mercury electrode with extremely slow drop rate (ca. 60 sec./drop) and the potentiometer-recorder for ordinary chronopotentiometry was investigated. Also an ordinary chronopotentiometry with stationary electrode but with decreasing current scan was studied. These methods show a similar effect to that of the present method described above. Therefore these three methods, all employing varying current density, would give potential values for analytical instrumentation. The detailed discussion will be presented later.

The authors gratefully acknowledge to Professor I. M. Kolthoff of the University of Minnesota for his kind interest and suggestion on the subject at the time of the International Symposium on Modern Electrochemical Methods of Analysis held at Paris in 1957. The authors also wish to thank Mr. A. Saito for his cooperation in this work.

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A New Colorimetric Method for the Determina tion of Beryllium with Neo-thorone

By Shozo Shibata, Fukuo Takeuchi and Teiichi Matsumae

(Received August 22, 1958)

In order to determine a few micrograms of beryllium, a new colorimetric method has been developed based upon the stable violet-colored beryllium complex of Neothorone[1] (*o*-arsonophenylazo-chromothropic acid)[2] in an aqueous solution.

1) S. Shibata and T. Matsumae, This Bulletin, **31**, 377 (1958).
2) V. Kuznetsov, *Compt. rend. acad. Sci. U. S. S. R.*, **31**, 898 (1941).

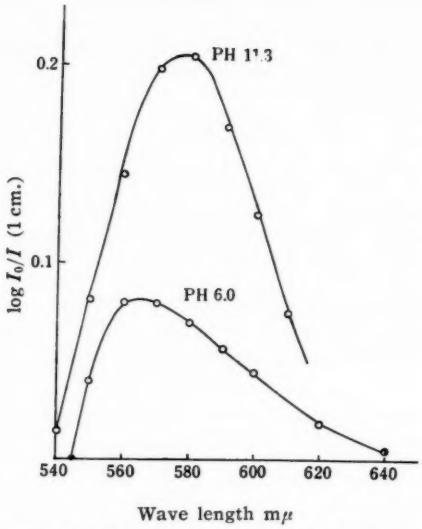

Fig. 1. Absorption spectra of Neo-thorone-beryllium complex at pH 6.0 and 11.3.

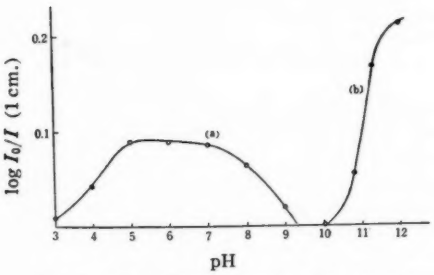

Fig. 2. The effect of pH on the absorbance (a, at 570 mμ; b, at 580 mμ).

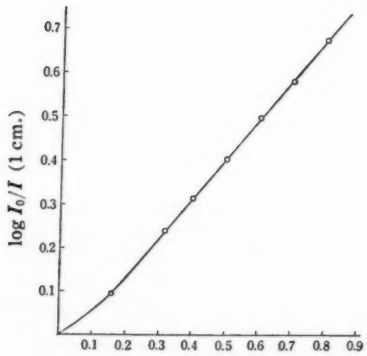

Fig. 3. Absorbance-concentration curve of beryllium (570 mμ, pH 6.0).

Absorption spectra of the complex are presented in Fig. 1. These curves were measured in a buffered solution of pH 6.0 and 11.3 respectively. The violet colored complex of beryllium was formed by adding several micrograms of beryllium and 1.0 ml. of 0.1% reagent solution to a 25 ml. volumetric flask and diluting to the mark with a buffered solution of pH 6.0. Color was developed within five minutes and its stability was found to be quite adequate for the determination of beryllium, the absorbance being proportional to beryllium concentration in the range from 0.15 p.p.m. to at least 1 p.p.m. (Fig. 3). At 570 mμ, the molar extinction coefficient is about 7,160, the sensitivity being 0.03 μg. Be/cm^2 corresponding to log I_0/I=0.001.

In a strong alkaline solution (pH 11.0~12.0), the absorbance becomes very high. The relationship between absorbance and pH of solution is shown in Fig. 2. The results given in Fig. 2 indicate the presence of two species of neo-thorone-beryllium complex. At pH 11.3, the absorbance is proportional to beryllium concentration in the range from 0 to 0.15 p.p.m. (Fig. 4). At

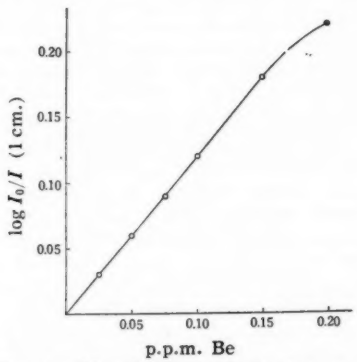

Fig. 4. Absorbance-concentration curve of beryllium (580 mμ, pH 11.3).

580 mμ, the molar extinction coefficient is about 10,000, the sensitivity being 0.002 μg. Be/cm^2 corresponding to log I_0/I=0.001.

Thus these two methods appear to offer a good basis for the colorimetric determination of microgram amounts of beryllium. Details of the study will be published later.

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On the First Ionization Energies of Heterocyclic Molecules

By Kogoro MAEDA

(Received August 23, 1958)

In the present note the author wishes to show some results of semi-empirical estimation of the first ionization energies of π-electrons in heterocyclic molecules. The evaluation of these values was carried out according to Hush and Pople's method[1] in which electron interaction was taken into account explicitly.

The values of the integrals, resonance β_{pq}, one center electron repulsion $(pp|pp)$, and two center electron repulsion $(pp|qq)$, were taken from Pariser and Parr's paper[2], those of the first integrals for non-neighboring atoms p and q being neglected; it was assumed that Coulomb integral α_{cp} and resonance integral β_{pq} were identical for all carbon atoms and carbon-carbon bonds in the different molecules (see Table I) respectively. The interatomic distances in the molecules considered here were taken from electron diffraction data by Schomaker and Pauling[3].

With respect to the molecular orbitals, those obtained by the Hückel method were used as approximations, because in practice it is rather tedious to solve the self-consistent equation completely. The parameters of energy integrals in the Hückel method were chosen from Orgel et al.[4], except for all carbon atoms and the sulfur atom in thiophene. For the carbon the common value was taken (so-called α_c), and for the sulfur Longuet-Higgins' idea was employed. Furthermore electron densities of the carbon atoms were assumed to have equal values of those in alternant hydrocarbons, and the Coulomb integral was chosen to make the ionization energy computed for benzene equal to the experimental value.

In Table I are given the results, which are in good agreement with experiment, although many approximations were necessary through the calculation. It has been proposed that in pyridine and pyrazine the ionization energies of these compounds are largely determined by the non-bonding electrons on the nitrogen atom, from the studies of their methyl derivatives by electron impact method[6] and from the results of self-consistent calculation on these electrons[7]. The energy interval of bombarding electrons, however, should be examined in the electron bombarding study, especially when the highest-occupied π-level may exist near nonbonding σ-levels. Details will be further discussed in subsequent publications.

TABLE I

RESULTS OBTAINED OF THE FIRST IONIZATION ENERGIES OF π-ELECTRONS, eV.

	calc.	obs.
benzene	9.24	9.24[8]
pyridine	9.45	9.76±0.05[9], 9.23[10]
pyrazine	9.90	10.01±0.02[6]
pyrrole	9.03	8.97±0.05[9]
furan	8.94	9.00±0.10[9], 8.89[10]
thiophene	9.34	9.1±0.2[9]

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1) N. S. Hush and J. A. Pople, *Trans. Faraday Soc.*, **51**, 600 (1955).
2) R. Pariser and R. G. Parr, *J. Chem. Phys.*, **21**, 767 (1953).
3) V. Schomaker and L. Pauling, *J. Am. Chem. Soc.*, **61**, 1769 (1939).
4) L. E. Orgel, T. L. Cottrell, W. Dick and L. E. Sutton, *Trans. Faraday Soc.*, **47**, 113 (1951).
5) H. C. Longuet-Higgins, ibid., **45**, 173 (1949).
6) I. Omura et al., This Bulletin, **30**, 633 (1957).
7) T. Nakajima and A. Pullman, *Compt. rend.*, **246**, 1047 (1958).
8) In the present report, this value was adopted as the most probable one; cf. J. D. Morrison, *J. Chem. Phys.*, **22**, 1219 (1954), K. Watanabe, ibid., **22**, 543 (1954).
9) H. Baba et al., This Bulletin, **29**, 521 (1956).
10) K. Watanabe, *J. Chem. Phys.*, **26**, 542 (1957).

Paramagnetic Resonance in Coals

By Hidemasa HONDA, Kazuo CHITOKU, Yasaburo YOKOZAWA and Keniti HIGASI

(Received September 13, 1958)

Paramagnetic resonance absorption in coals has been attributed by Bennet, Ingram and others[1] to free radicals which are associated with a broken bond in the condensed aromatic structure. Contrary to this, facts have been discovered which appear to suggest another possibility. First, pyridine extracts of coal show less

1) J. E. Bennet, D. J. E. Ingram and J. G. Tapley, *J. Chem. Phys.*, **23**, 215 (1955).

TABLE I
ANALYSIS OF COALS USED

Source	Moisture	Ash	d. a. f. base %		O+N+S + error
			C	H	
Rich Rum	0.68	7.90	82.95	5.77	11.28
Mathics Bituminous	0.78	8.17	83.16	6.17	10.67
Monongalia	0.58	5.72	83.31	5.97	10.72
Elkhorn	0.82	3.13	83.39	5.80	10.81
Jerry Fork	0.92	5.81	84.46	5.55	9.99
Grichton	0.72	3.79	86.20	5.33	8.47
Bergoo	0.53	5.29	86.60	5.37	8.03
Denise	0.84	5.90	88.95	4.79	6.26
Maryland Shapy	1.00	9.19	89.08	5.03	5.89
Mammoth Anthracite	1.05	13.68	89.44	3.95	6.61
Indian Ridge	0.11	5.24	90.11	5.14	4.75
Primrose Anthracite	0.43	9.35	90.32	4.13	5.55
Nakagō (Japan)	13.32	3.96	74.33	5.26	20.41
Yūbari (Japan)	1.13	2.67	84.85	6.16	8.99
Yatake (Japan)	1.11	2.74	89.55	4.86	5.59
Uonuki (Japan)	1.68	1.80	91.16	3.97	4.87

but distinct paramagnetic resonance[2]. If the paramagnetic substance be actually due to a broken bond, possibly it might be destroyed by some physical changes. Secondly, both violanthrone and violanthrene[3], which have similar aromatic chemical composition to coal but no broken bonds show distinct paramagnetic resonance absorption.

Recently a suggestion has been advanced that the resonance absorption in coals may be due not to the unpaired electrons present in free radicals or broken bonds but to the unpaired π electrons in the triplet state[4]. On the other hand, Austen and Ingram et al. have emphasized again that the resonance absorption in coals is due to the free radicals formed by breakage of bonds round the edge of the carbon clusters or originated in the formation of defects in the ring packing and not to the exited triplet state[5]. Still evidence is lacking to make a decisive choice between the two views. In the present paper a trial will be described which was done in the above connection.

Experimental Material and Method

Twelve American coal specimens are kindly provided by Dr. K. Inouye who is staying at Pennsylvania State University. All of these samples have been kept in the fresh state: During the storage and transportation the samples were sealed in an atmosphere of nitrogen, and contact with air was avoided as far as possible. Four Japanese specimens were chosen for the sake of comparison. Chemical analysis of the samples used is shown in Table I.

The observation of resonance absorption were carried out for each specimen treated in two different ways: (a) a sample sealed under vacuum and (b) the same kept in air. The measurements were made by use of a microwave of 3.2 cm. wave-length at room temperature[6]. The signal intensity was determined in comparison with diphenylpicrylhydrazil (DPPH). Paramagnetic susceptibility per gram thus obtained is recorded in Table II.

TABLE II
PARAMAGNETIC SUSCEPTIBILITY OF COAL
(10^{-6}c g s per gram)

Specimen	In vacuum	In air
Rich Rum	0.67	1.36
Mathics Bituminous	0.58	0.77
Monongalia	0.70	0.87
Elkhorn	1.03	0.73
Jerry Fork	0.93	0.60
Grichton	1.12	0.93
Bergoo	0.64	0.78
Denise	1.86	1.57
Maryland Shapy	1.16	2.02
Mammoth Anthracite	4.20	2.55
Indian Ridge	1.16	1.68
Primrose Anthracite	3.65	2.36
Nakagō (Japan)	0.40	0.37
Yūbari (Japan)	0.28	0.82
Yatake (Japan)	1.23	2.32
Uonuki (Japan)	3.90	3.39

2) Y. Yokozawa, I. Miyasita, M. Kugo and K. Higasi, This Bulletin, **28**, 536 (1955).
3) Y. Yokozawa and I. Tatsuzaki, *J. Chem. Phys.*, **22**, 2087 (1954).
4) H. Honda and K. Ōuchi, *Fuel*, **36**, 159 (1957).
5) D. E. G. Austen and D. J. E. Ingram, *Brennstoff Chemie*, **39**, Sonderausgabe, 25 (1958); D. E. G. Austen, D. J. E. Ingram and J. G. Tapley, *Trans. Faraday Soc.*, **54**, 400 (1958).
6) Y. Yokozawa and I. Tatsuzaki, *Bull. Res. Inst. Appl. Elec.*, **6**, 111 (1954).

Results and Discussion

As pointed out by Ingram[7] the action of oxygen on the paramagnetic absorption was found to be important. If broken bonds be responsible for paramagnetic resonance, one may expect that the admission of air would destroy free radicals and the signal intensity in air would become weaker than that in vacuum. The result of experiment was more complicated than expected*. In half of fresh coals the absorption intensity decreases in the presence of the oxygen, while in the remaining half it even increases. If the fresh coals actually be abundant in free radicals of broken bonds, the trends of this oxygen influence would be hard to understand.

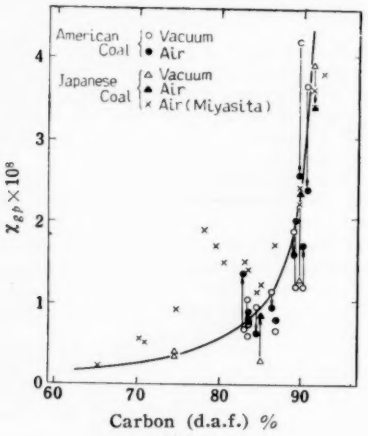

Fig. 1

In Fig. 1 the paramagnetic susceptibility of coal is plotted against the carbon content (d. a. f.). For the sake of comparison, Miyasita's[8] values are recorded in the same graph—in the latter case the samples were dried at 105°C and were measured mostly in air. Again it will be seen that there is no abnormality in resonance intensity for the fresh American coals.

Remembering that the "freshness" of coal is a matter of comparison, one may not be entitled to deny the theory of broken bonds solely on the basis of the present observation. Still it strongly suggests that further critical examination on that theory should be desirable.

The authors are grateful to Drs. K. Inouye and W. Spackman for fresh specimens which are representatives of the American eastern coals. Thanks are also due to Professor G. Takeya and Dr. M. Kugō for discussion.

Resources Research Institute, Kawaguchi Saitama, and Research Institute of Applied Electricity, Hokkaido University Sapporo

7) J. E. Ingram, *Disc. Faraday Soc.*, **19**, 179 (1955).

*) Measurements were made also on the oxidized products of four specimens obtained by heating at 150°C for 1, 2 and 3 hours. Each of them was found to be paramagnetic and the signal intensity was of the same order with that of the original sample.

8) I. Miyasita, Dissertation of Hokkaido University for the degree of doctor of science (1957); see also I. Miyasita and Y. Yokozawa, *Bull. Res. Inst. Appl. Elec.*, **7**, 162 (1955).

Solubility of Ethylenediaminetetraacetic Acid in Some Aqueous Solutions

By Yukichi Yoshino, Akira Iguchi, Masuo Kojima and Kunihiko Mizumachi*

(Received September 9, 1958)

In the course of study on the use of ethylenediaminetetraacetic acid as eluant for ion-exchange chromatography, it was found necessary to investigate its solubility behavior. No such data being available**, the authors measured the solubility of free acid, H_4Y, under various conditions.

The free acid was recrystallized thrice from an ammoniacal solution by adding dilute hydrochloric acid and then dried at 110°C for several hours. Chemical analysis by titration with standard zinc solution using Eriochrome Black T as indicator proved the sample was pure enough for this purpose. The free acid thus obtained was shaken with various media in a thermostat until equilibrium was attained. Then a portion of the saturated solution was taken by means of a pipette attached with cotton filter and the concentration of the free acid was determined by titration with 0.005 M standard zinc solution at pH 10 using the same indicator. All other chemicals were of guaranteed grade and were purified by distillation or recrystallization, if necessary.

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** Solubility of disodium salt was reported by N. J. Blaedel and H. T. Knight, *Anal. Chem.*, **26**, 743 (1954).

TABLE I

TEMPERATURE VARIATION OF THE SOLUBILITY OF ETHYLENEDIAMINETETRAACETIC ACID IN WATER

Temperature (°C)	Concentration of EDTA (H_4Y) (10^{-3}mol./l.)
2.7	0.566*
25.0	1.153
30.0	1.372
35.0	1.660
40.0	1.990
45.0	2.350
100	32.1

* Preliminary data of less accuracy.

Table I shows the temperature dependence of the solubility of ethylenediaminetetraacetic acid in water. Within the range of temperature between 25°C and 45°C the plot of the logarithm of solubility against the reciprocal of absolute temperature gave straight line and from its slope the heat of solution was found to be 6.76 kcal./mol. (endothermic) in the temperature range of our experiment.

TABLE II

SOLUBILITY OF ETHYLENEDIAMINETETRAACETIC ACID IN POTASSIUM CHLORIDE SOLUTIONS AT 30°C

Concentration of potassium chloride (mol./l.)	Concentration of EDTA (H_4Y) (10^{-3}mol./l.)
0.00	1.372
0.01	1.509
0.04	1.636
0.09	1.781
0.16	1.901
0.20	1.990
0.50	2.330
1.00	2.650

The effect of the ionic strength on the solubility of the acid was determined in solutions of varying concentration of potassium chloride. The results are given in Table II. As is expected from the amphoteric nature of ethylenediaminetetraacetic acid, the solubility curve plotted against hydrochloric acid concentration passed through a minimum which is illustrated in Fig. 1.

The solubility of the acid in water-ethanol mixture was decreased with increasing of ethanol contents, as was shown in Fig. 2.

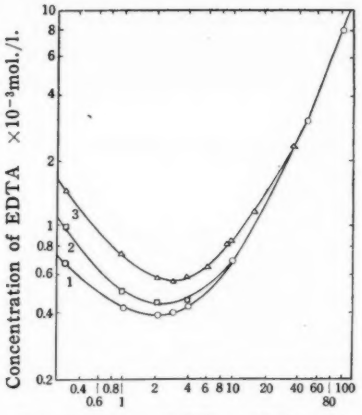

Fig. 1. Relationship between the solubility of EDTA (H_4Y) and the molarity of HCl at 30°C.
1. The aqueous solution of HCl.
2. The aqueous solution of HCl-KCl. (μ=0.10)
3. The aqueous solution of HCl-KCl. (μ=0.50)

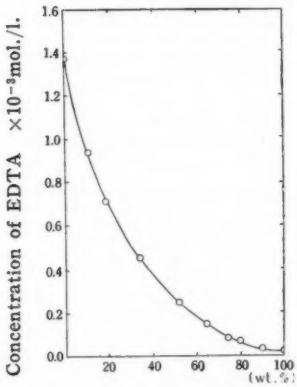

Fig. 2. Solubility of EDTA (H_4Y) in ethanol-water mixtures (at 30°C).

Details of the experiment will be reported elsewhere.

A part of the expense of this study was defrayed by a grant-in-aid from the Ministry of Education.

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